BARK BEETLE BEHAVIOR, ATTRACTION AND PERFORMANCE IN NATURAL AND MANAGED PONDEROSA FORESTS OF ARIZONA

FINAL REPORT

USDA Agreement 05-PA-11221615-161
BETWEEN THE USDA ROCKY MOUNTAIN RESEARCH STATION
RESEARCH WORK UNIT NUMBER RMRS-4156

AND

ARIZONA BOARD OF REGENTS, FOR AND ON BEHALF OF
NORTHERN ARIZONA UNIVERSITY SCHOOL OF FORESTRY
Dr. Richard Hofstetter & Dr. Mike Wagner

TITLE: Bark Beetle Behavior, Attraction and Performance
in Natural and Managed Ponderosa Forests of Arizona'

Principle Investigator: Dr. Richard W. Hofstetter, School of Forestry, N.A.U.

Co-Principle Investigators: Carl Edminster (Rocky Mountain Research Station-4156); Forest Health Protection Entomologists John Anhold & Joel McMillin (Flagstaff); University of Arizona Forest Health Specialist Tom DeGomez (Flagstaff); Northern Arizona University (NAU) Professor Mike Wagner (Flagstaff).

I. Project Overview

The purpose of this study is to (1) provide regionally appropriate data that supports or refutes the effectiveness of traditional silvicultural strategies (e.g., thinning) to reduce pine bark beetle damage, (2) identify chemical lures that effectively attract and monitor western and southern pine beetles, and (3) understand how ambient temperature influences beetle flight activity in southwestern ponderosa pine forests.

The first objective emphasizes how changes in stand density and/or composition influence chemical composition of resin and crystallization rate, nutritional quality of phloem to beetles and their associated fungi, and microclimate. We address stand-level differences in tree mortality by comparing tree susceptibility to beetles across thinning treatments. Interspatial distance between trees within stands and basal area likely affect tree resistance characteristics and bark beetle landing behavior. The evaluation of the effectiveness of stand-level thinning will contribute to the development of silvicultural methods and standards that will protect ponderosa forests from area-wide bark beetle outbreaks.

The current knowledge of the pheromones of many of the southwestern bark beetles come from studies performed in the northwest and southeast U.S. Many of the commercial lures used as attractants utilize host plant volatiles in combination with known beetle emitted compounds. However, there is considerable geographic variation in the profiles of volatiles among and between ponderosa pine populations. Regional differences in ponderosa chemical profiles likely influence the preference of bark beetles at the local level. Consequently, the identification of effective host volatiles used as pheromone lure synergists may increase the effectiveness of trapping for beetles in

Arizona. Trap catches are of critical importance in managing and monitoring the activity of bark beetles in all forest ecosystems, including ponderosa pine forests in Arizona.

Spring and fall flight periods of beetles are believed to be driven primarily by temperature. By monitoring trap catches in conjunction with local climatic patterns, we can identify optimal temperature ranges of beetle activity and improve our understanding of temperature-dependent behavior of many bark beetle species in the southwestern U.S. This knowledge will assist in the development of IPM strategies to protect critical habitat and improve timing of preventative spray treatments to protect trees. In addition, this information will help predict the potential range expansion of beetle populations due to broad changes in climatic patterns, such as global warming.

II. Accomplishments and Significance

Objective 1 [Thinning guidelines to prevent ponderosa pine bark beetle outbreaks in the Southwest]

It was our interest to provide regionally appropriate data that supports or refutes the conventional wisdom that stand density regulation is the most effective strategy to prevent pine bark beetle damage to Southwestern ponderosa pine forests. There is a growing misunderstanding of the effectiveness or lack of effectiveness of silvicultural management of bark beetles. The objectives of this study were to provide a (1) regional assessment of the effects of thinning on bark beetle landing behavior, flight activity, and reproduction, and (2) regional validation of the relationship between ponderosa pine stand density and western bark beetle species.

We monitored beetle movement within six replicated stand basal area treatments (30, 60, 80, 100, 120, 150 ft^2/acre) using sticky traps on trees and passive window traps between trees. Sticky and passive traps were located in Taylor woods between May – October 2005. The window and funnel traps assessed bark beetle flight patterns, while sticky traps assessed beetle landing behavior. We assessed bark beetle reproduction by baiting trees within each stand class and measuring the number of entry and exit holes and gallery length per area of bark.

Beetle landing behavior and movement through stands was influenced by stand basal area. In general, more bark beetles and predators were captured (in passive traps) in stands of low basal area (i.e. stands that are more open). Beetles appear to select trees prior to landing rather than land randomly and choose trees after landing. Bark beetles were rarely captured on trees, but predators were periodically captured throughout the study on sticky traps. In general, more predators were captured on trees in stands of lower basal areas. More bark beetles and predators were captured in passive traps than on sticky traps.

Bark beetle performance among stands was influenced by stand basal area. Trees in higher basal stands had higher densities of beetles as measured by attacks, offspring, and galleries. However, reproductive performance as reflected by the ratio of offspring to attacks was greater in stands with lower basal areas. High beetle densities may have resulted in poorer reproduction in stands of high basal area.

With regard to tree resistance to bark beetles, trees in stands of low basal area had larger resin reserves and thus may have a greater chance of survival from low beetle attack densities. We are performing chemical analyses of resin (terpenes, phenolics) and

phloem nutrients from trees within the different thinning treatments. Preliminary results also indicate that predator activity is greater in stands will lower basal area. This information will be used to improve stand risk/hazard ratings and tools for evaluating potential silvicultural and management options to develop optimal strategies to protect critical habitats.

Objective 2 [Influence of host volatiles as pheromone lure synergists for southern pine beetle and western pine beetle in Arizona]

The current standard lure for the western pine beetle (*Dendroctonus brevicomis*) includes the host volatile myrcene as a pheromone-synergist. However, considerable geographic variation exists in monoterpene profiles among and between ponderosa pine populations which may influence the relative effectiveness of these lures. In Arizona, α-pinene is more abundant in pines than myrcene and we postulated it would be a better synergist to attract beetles. The southern pine beetle (*D. frontalis*) is a common bark beetle pest in Arizona but little is known about the synergistic effects of monoterpenes as attractants in Ponderosa pine forests.

The purpose of this study was to (1) experimentally test the current commercially available pheromone lures and lures with modified host volatiles for southern pine beetle and western pine beetle in Arizona and (2) determine whether *D. frontalis* is more attracted to the western pine beetle lure than the southern pine beetle lure. The following lures were tested using Lindgren funnel traps: (1) control (trap without bait), 2) frontalin, 3) frontalin + myrcene, 4) frontalin + α-pinene [25% (-) α-pinene + 75% (+) α-pinene blend], 5) exo-brevicomin + frontalin + myrcene, 6) exo-brevicomin + frontalin + α-pinene blend, and 7) exo-brevicomin + α-pinene blend. Trapping arrays were set up in 10 locations in ponderosa pine forests within 100 miles of Flagstaff, Arizona. Traps were collected weekly from May and July 2005. All insect were counted and identified to species.

The majority of insects (93%) collected in the traps were *D. frontalis* and *D. brevicomis*. The best lure to attract *D. frontalis* and *D. brevicomis* in Arizona was the combination of Frontalin, *exo*-brevicomin and α-pinene. The replacement of myrcene with α-pinene attracted twice as many *D. brevicomis* and *D. frontalis* than the traditional lures. *D. frontalis* appears to be significantly attracted to *exo*-brevicomin component. The traditional *D. frontalis* lure (frontalin + terpene) was not very effective. *Temnochila* was the most abundant predator trapped and was most attracted to lures containing *exo*-brevicomin. *Elacatis*, a common predator in Arizona, was most attracted to the Frontalin, *exo*-brevicomin and α-pinene combination. For *Elacatis*, α-pinene appears to be an important component for attraction. *Enoclerus* was rarely caught in our traps.

The chemical composition of hosts likely influences speciation and the evolution of aggregation pheromones in bark beetles. An explanation for the attraction of *D. frontalis* to the western pine beetle lure remains unclear. Preliminary studies show that *D. frontalis* are potentially attracted to the *endo*-brevicomin impurities (~3%) in the *exo*-brevicomin lure. Our results also indicate that predators use local terpenes to find potential prey. This is especially evident with the predator *Elacatis*. Our study results will allow for more effective (optimal) trapping for southern and western pine beetles, improve monitoring of beetle populations and management at landscape level, and lead to improved lures and reduced trap catches of predators.

Objective 3 [Flight temperature thresholds for Southwestern ponderosa pine bark beetles.]

Predicting when bark beetle flights initiate in the spring is crucial for land managers to improve timing of control and removal tactics to reduce beetle effects. Little research has been done on seasonality of bark beetles in Southwestern ponderosa pine forests. We were interested in determining temperature threshold and/or degree day accumulation necessary for initiation of bark beetle flights in Southwestern ponderosa pine forests and creating a working model that will predict first bark beetle emergence/flight each year.

Bark beetle flight and temperature data were collected over a 2 year period (Jan. 2002 – Dec. 2003). Traps were set up in Centennial forest, approximately 10 km west of Flagstaff, 7000 feet elevation. Ten clusters of five traps were baited with lures for both *Ips* and *Dendroctonus* beetles. Air temperatures were recorded from a data logger located within 2 miles of each trap cluster. In 2005 we used three clusters of three traps baited with lures for *Ips* or *Dendroctonus* beetles located within 50 km of Flagstaff. Two sets of traps were located at approximately 9000 feet elevation and the remaining trap cluster was at 7000 feet elevation. Each trap cluster had a temperature data logger located within 50 feet of the traps.

Incorporating our temperature data into beetle development models, we predict that *D. frontalis* has 2.7 to 2.8 generations per year in the Flagstaff area. This is fewer generations per year than that found in any area of the southeast U.S. Generation models for other bark beetles in Arizona are still in progress.

In general, threshold temperatures were a better predictor of flight initiation than degree day models for 2002 and 2003. For all species (except Elacatis) degree days until capture were different between 2002 and 2003. First flight for most species in 2002, 2003 occurred when air temperatures reached 20C. *Elacatis*, *Ips pini* and *D. adjunctus* appeared to initiate flight at slightly cooler temperatures (around 15C). The bark beetle predator, *Temnochila*, was not captured until temperatures exceeded 25C. In 2005 results were similar for threshold response with *I. pini* and *D. adjunctus* captured earlier and at lower temperatures than other bark beetle species. Data on *Elacatis* was not available for 2005. *Temnochila* were captured in 2005 when temperatures approached 20C. Temperature data loggers were not installed early enough in 2005 to adequately assess degree day accumulation at each location. Daytime maximum temperatures in our area did not appear to exceed the upper temperature limits for flight activity for the majority of species.

This model will clarify the optimal temperature ranges of beetle flight and improve the timing of preventative spray treatments to protect trees in critical habitats and improve the effects of thinning treatments on beetles at the local scale. The model will also predict potential changes in beetle population dynamics in relation to global climate changes.

We are still working with different modeling techniques to best predict beetle flight patterns. We are also still analyzing trap data from other locations from 2005 to validate our model.

Publications and Outreach

Research results have been presented at multiple scientific meetings: The Annual Forestry Expo in Morelia Mexico November 2005, The Entomological Society of America National Meeting in Ft. Lauderdale, Florida December 2005, North American Forest Insect Work Conference in Ashville NC May 2006, and Western Forest Insect Work Conference in Boise ID March 2007.

Scientific publications are in preparation and will be submitted during spring and summer 2007. The articles are attached at the end of this document. These publications include:

1. Hofstetter, R.W., S. Martinson, M.L. Gaylord and M. Wagner. Differences in attraction of primary and secondary bark beetles and their predators to terpene levels and pheromone combinations. Agriculture and Forest Entomology.
2. Gaylord, M., K. Williams, R.W. Hofstetter, J. McMillin, T. DeGomez, & M.R. Wagner. Flight temperature thresholds for southwestern ponderosa pine bark beetles. Environmental Entomology.
3. Hofstetter, R.W., D.S. Pureswaran, & B. Sullivan. Attraction of the southern pine beetle, *Dendroctonus frontalis* to pheromones of the western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Curculionidae: Scolytinae) in an allopatric zone. J. Ecol. Ent.
4. Hofstetter, R.W., Z. Chen, M. Gaylord, J. McMillen & M. Wagner. Synergistic effects of the attractants a-pinene and exo-brevicomin on the southern and western pine beetle and associated predators in Arizona. J. Appl. Entomology.

Implications

This research has implications in better understanding the behavior and impacts of bark beetles in managed ponderosa pine forests. This research shows that environmental factors such as climate and inter-tree spacing impact beetle reproduction, behavior and population dynamics. Additionally, our results show that management of forests can impact these factors.

Journal: Journal of Applied Entomology

Synergistic effects of the attractants α-pinene and *exo*-brevicomin on the southern and western pine beetle and associated predators in Arizona.

Hofstetter, R.W. [1], Z. Chen[1], M. L. Gaylord[1], J.D. McMillin[2] and M. R. Wagner[1]

[1] School of Forestry, Northern Arizona University, Flagstaff, AZ

[2] USDA Forest Service, Forest Health Protection, Flagstaff, AZ

Corresponding author: Richard W. Hofstetter

Address: School of Forestry, Northern Arizona University, Box 5018, Flagstaff Arizona 86011-5018, USA

Telephone: 928-523-6452; FAX: 928-523-1080

Email: Rich.Hofstetter@nau.edu

Abstract: The bark beetles, southern pine beetle (*Dendroctonus frontalis*) and western pine beetle (*D. brevicomis*) cause significant mortality to pines in the southern and western United States. The effectiveness of commercial lures at capturing these beetles in Arizona has not been tested and may vary from other regions of their distribution. We conducted field experiments using baited Lindgren funnel traps to investigate (1) if *D. frontalis* is more attracted to the standard commercial lure for western pine beetle (frontalin + *exo*-brevicomin + myrcene) than the standard commercial lure southern pine beetle (frontalin + terpene blend), (2) whether replacement of myrcene with α-pinene in the lures changes trap catches of bark beetles and associated competitors and predators, and (3) whether the attraction to these lures varies across ponderosa pine (*Pinus ponderosa*) forests throughout Arizona. *Dendroctonus frontalis*, *D. brevicomis* and the predator *Temnochila chlorodia* were most attracted to lures with *exo*-brevicomin (i.e. western pine beetle lure). The replacement of the myrcene component with α-pinene in the western pine beetle lure resulted in the capture of twice as many bark beetles and twice as many of the predator *Elacatis* spp. In seven out of nine locations throughout Arizona, the western pine beetle lure with α-pinene was more attractive than the lure with myrcene or a terpene blend. These results suggest that the western pine beetle lure with α-pinene rather than myrcene is a more effective lure to capture *D. brevicomis* and *D. frontalis* in Arizona. However, geographic variation in attractiveness to these lures is evident even within this small region of the beetles' distributions.

Keywords: *Dendroctonus, frontalis, brevicomis, Temnochila, Enoclerus, Elacatis*, pheromone, lure, bark beetle, *Pinus ponderosa*, Southwest

1 Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) use pheromones and plant compounds to focus beetle attacks on host trees (i.e. mass aggregation) (Wood 1973, 1982). For species that colonize living trees, it is important to cooperate with conspecifics to overcome tree defensive mechanisms (Raffa 2001 and references within). When two or more species colonize the same tree, species-specific pheromones may

promote successful multi-species aggregations (Svihra et al. 1980, Smith et al. 1990) and serve to partition the resource and minimize interspecific competition (Lanier and Wood 1975, Light et al. 1983; Rankin and Borden 1991). Cross attraction by species successfully colonizing living trees infers a potential mutalism(s), while strong deterrence of heterospecific pheromones suggests an antagonistic relation(s) between species. In the genus *Dendroctonus,* females beetles initiate attack, excavate galleries under the bark, and release aggregation pheromones that are attractive to both sexes (Borden et al. 1986; Raffa et al. 1993). Males beetles seek females within trees and produce pheromones which further facilitate aggregation. Once beetles mate and females begin to oviposit eggs, antiaggregation pheromones are produced by one or both sexes to reduce potential negative impacts from conspecifics and interspecifics within the host tree (Borden et al. 1986; Raffa et al. 1993).

In Arizona USA, two aggressive bark beetles species, *Dendroctonus frontalis* and *D. brevicomis* are sympatric, cohabit the same trees (Breece et al., in review), and show identical seasonal patterns in abundance in ponderosa pine forests (Sanchez-Martinez & Wagner 2002, Gaylord et al. 2006). Since 2000, these species have caused significant mortality to ponderosa pine forests (USDA, Forest Service R-3, Forest Health Protection, Insect and Disease Condition Reports 2001-2006). Previous trapping efforts for these species in northern Arizona have used only the Western Pine Beetle lure (*exo*-brevicomin, frontalin and myrcene) or the Southern Pine Beetle lure without a terpene component (only frontalin) (Sanchez-Martinez & Wagner 2002, Gaylord et al. 2006). In the southeast United States where *D. brevicomis* does not occur, *exo*-brevicomin reduces or has no affect on *D. frontalis* captures within beetle infestations (Payne et al. 1977; however see Pureswaran et al., in press). In Arizona, where *D. frontalis* and *D. brevicomis* occur in sympatry, relative attraction to *exo*-brevicomin by *D. frontalis* in the presence of terpenes is unknown

Commercially produced pheromones used for beetle-monitoring are generally species specific but are not regionally tested (Byers 1995) and therefore, may not accurately assess population abundances and the relative abundance of two or more species (Billings and Bryant 1983). Most commercially available lures result from studies that took place in only a few locations throughout the beetle's distribution. For

instance, studies in California and Oregon suggest that *D. brevicomis* uses the terpene myrcene to synergize its pheromone (Bedard et al. 1969, Pitman and Vite 1971, Sturgeon 1979, Byers 1982). These studies have contributed to the production of the Western Pine Beetle (WPB) lure which uses the terpene myrcene, as a synergist, with *exo*-brevicomin and frontalin. However, the considerable geographic variation in monoterpene profiles of host trees across its geographic range (Smith 2000) may influence the host location behavior of *D. brevicomis* (Sturgeon 1979) as well as other bark beetles (Wood 1982, Raffa 2001). For instance, while the monoterpene component of ponderosa pine in Arizona, USA is extremely variable, in general, α-pinene is the predominant component while myrcene comprises very little of the resin of these trees (Table 1).

Bark beetle pheromones and host tree terpenes are exploited by predators and competitors to locate food resources (Wood 1982, Poland & Borden 1997, 1998, Zhou et al. 2001, Aukema & Raffa 2005). Regional differences have been found among predator preferences and the pheromones emitted by bark beetles (Herms et al. 1991, Seybold et al. 1992, Raffa & Dahlsten 1995). The intensity of attraction of predators by the lures used to attract *D. frontalis* and *D. brevicomis* in Arizona is not well known. One goal of using bark beetle pheromones for monitoring or other management strategies is to maximize bark beetle catches, while minimizing predator catches (references).

We conducted two field trapping experiments to investigate the attractiveness of current commercially available pheromone lures and lures with modified host volatiles for *D. frontalis*, *D. brevicomis* and their associated insects in north-central Arizona. Objectives for the first experiment were to determine if *D. frontalis* is more attracted to the WPB lure than the SPB lure, and whether the replacement of myrcene with α-pinene changes trap catches of bark beetles, competitors or predators. The objective for the second experiment was to determine whether capture rates and lure preferences vary across pine forests throughout Arizona. For this experiment, we added an additional lure, frontalin + *exo*-brevicomin + terpene blend (modified synthetic re-creation of the terpene component of Arizona ponderosa pine, Table 1) and compared its attraction to beetles and associated insects to the WPB lure with myrcene or α-pinene. Improved lures for *D. brevicomis* and *frontalis* will allow for more effective monitoring of these species throughout this region and may lead to a separate lure for each species. Predator

responses... Better understanding of aggregation behavior of sympatric aggressive bark beetles species.

2 Material and Methods

2.1 Experiment 1 – comparison of commercial lures and terpene alternatives

Trapping Experiment 1 was conducted to compare the relative attractiveness of commercially available pheromone lures with modified lures to *D. frontalis, D. brevicomis* and associated insects in ponderosa pine stands within the Coconino National Forest of north-central Arizona. Ten blocks of seven 8-unit funnel traps (Phero Tech Inc., Delta, BC, Canada; Lindgren 1983) were arranged in a circular pattern, with 50 m between adjacent traps. Blocks were separate by a minimum of 500 m and located within 200 km of Flagstaff, Arizona (lat/long). Each trap was hung on a metal conduit at least 3 m from the nearest tree with bottoms of traps 1 m above the ground. Each trap was randomly allocated to one of seven treatments and re-randomized each week. Lures were attached to the middle funnel of the trap. A 3x3 cm section of Spectracide® Bug Stop® pest strip (18.6% Dichlorvos, United Industries Corp., St. Louis, MO, US) was placed into each collection cup to kill captured insects and reduce predation by predatory insects. Trap catches were collected weekly for 6 weeks from May 30, 2005 to July 5, 2005. This design resulted in $n = 10$ blocks x 6 sample periods = 60 collections for each of the seven treatments. All insects, except Buprestidae and Cerambycidae, were identified to species or genus.

The following treatments were tested to determine their attractiveness to *D. frontalis*, *D. brevicomis* and associated insects: 1) blank control (no lure), 2) frontalin, 3) frontalin + myrcene, 4) frontalin + α-pinene [25% (-) α-pinene + 75% (+) α-pinene], 5) *exo*-brevicomin + frontalin + myrcene, 6) *exo*-brevicomin + frontalin + α-pinene [25% (-) + 75% (+)], and 7) *exo*-brevicomin + α-pinene [25% (-) + 75% (+)]. Frontalin was released at a rate of 2.0 ± 0.2 mg/day, myrcene at 12.0 ± 1.0 mg/day, and *exo*-brevicomin at 1.5 ± 0.2 mg/d with polyethylene bottles under field conditions (products from Phero Tech Inc., Delta, British Columbia; three component lure for *D. brevicomis*). The α-

pinene (SigmaAlrich) mixture was released from polyethylene bottles at a rate of 9.0 ± 0.2 mg/day in the field.

Analysis of variance was performed on each insect species for which more than five individuals were captured per trapping period. Data were analyzed using a randomized complete block design (SAS PROC MIXED; SAS *year*). Block and Block by Treatment interaction were considered random effects. In some cases, we tested combinations of predator or competitor species that showed significant treatment effects. Data were square-root transformed before analysis to reduce heteroscedasticity, although graphs and figures show raw means and standard errors. Where significant treatment effects occurred ($\alpha \leq 0.05$), differences were compared using pairwise *t*-tests on least squared means (Carmer & Swanson, 1973).

2.2 Experiment 2 – response to terpenes across Arizona

Trapping Experiment 2 was conducted to test the relative attractiveness of the myrcene, α-pinene and a terpene blend to *D. frontalis, D. brevicomis* and associated insects across a broader geographic range and variation in ponderosa pine. Traps were located at nine locations in ponderosa pine stands within National Forests across Arizona (Figure 1). Three lures were tested (*exo*-brevicomin + frontalin + myrcene, α-pinene, or terpene blend) at each location. Release rate of each component compound was similar to Experiment 1. The terpene blend was dispensed from 1.8ml polyethylene bottles with a release rate of 12 ± mg/day during the course of the study. Each lure was attached to a 12-unit funnel traps placed 30 m apart. At six locations one set of three traps were set up, while at three sites two sets of three traps separated by >5 km. Trap set up and use of the pest strip (insecticide) was similar to Experiment 1. Trap catches were collected after one to two weeks between June 15, 2006 and August 15, 2006. All insects were identified to species or genus. For some trap locations, we were unable to identify either sex or species of a proportion of *Dendroctonus* specimens due to poor condition of the insects within traps, and for these traps, a subset of beetles were used to calculate *Dendroctonus* species- and sex-ratios.

Analysis of variance was performed on each insect species for which more than 5 individuals were captured per trapping location. Data were analyzed using a randomized block design (SAS PROC MIXED; SAS *year*). Location and Location by Treatment interaction were considered random effects. Data were square-root transformed before analysis to reduce heteroscedasticity, although graphs and figures show raw means and standard errors. Where significant treatment effects occurred ($\alpha = 0.05$), differences were separated by pairwise *t*-tests on least squared means (Carmer & Swanson 1973).

3 Results

3.1 Experiment 1 – comparison of commercial lures and terpene alternatives

A total of 28,271 insects were captured representing more than 17 species (Table 2). *Dendroctonus* bark beetles accounted for 93% of total insects collected. The most abundant *Dendroctonus* species was *D. frontalis* followed by *D. brevicomis*, *D. valens*, *D. approximatus* and *D. adjunctus* (Table 2). The most abundant bark beetle predator was *Temnochila chlorodia* (Coleoptera: Trogositidae) followed by suspected predator *Elacatis* sp. (Coleoptera: Othniidae) and *Enoclerus* spp. (Coleoptera: Cleridae). High numbers of click beetles (Coleoptera: Elateridae) were also captured in traps (Table 2). Only *D. frontalis*, *D. brevicomis*, *D. valens*, *Elacatis* sp., and *T. chlorodia* were caught in sufficient numbers to warrant statistical analysis of sampling period and pheromone treatment. Trap catches of *D. frontalis* were relatively uniform throughout the experiment while all other abundant species varied considerably from week to week (Table 3). There was a significant Week x Lure interaction for several herbivore and predator species (Table 3).

Dendroctonus brevicomis and *D. frontalis* showed a significant attraction to the combination of *exo*-brevicomin, frontalin and α-pinene (Figure 2) over all other lure treatments. The replacement of the myrcene component of the WPB lure with α-pinene (BFP) attracted >2 times as many beetles of both species. Additionally, lures containing *exo*-brevicomin were significantly more attractive than lures without *exo*-brevicomin. *Dendroctonus valens* demonstrated a clear preference for lures containing terpenes (Table 3), while traps with just frontalin or the blank control caught significantly fewer *D.*

valens. Other phloeophagous and wood boring insects in the genera *Dendroctonus*, *Ips*, *Hylastes*, *Scolytus* and *Xyleborus* and in the families Buprestidae and Cerambycidae were collected too infrequently (< 1 individual per trap per week) to draw conclusions about their preferences for specific lures.

Temnochila chlorodia was the most abundant bark beetle predator (Table 2) and was most attracted to lures containing *exo*-brevicomin (Fig. 3A). This predator was also attracted to frontalin + α-pinene, but showed no more attraction to frontalin or frontalin + myrcene relative to its attraction to the blank control. Captures of the suspected predator *Elacatis* were strongly influenced by the presence of α-pinene. *Elacatis* was most attracted to the *exo*-brevicomin + α-pinene lure and the frontalin + *exo*-brevicomin + α-pinene lure (fig. 2B). The number of *Elacatis* captured in the blank control was not significantly different from the number captured in traps baited with frontalin, frontalin + α-pinene, frontalin + myrcene, or frontalin + exo-brevicomin + myrcene. Predators in the families Histeridae and Cleridae were collected too infrequently to draw conclusions.

3.2 Experiment 2

A total of 13,815 *D. frontalis* and *D. brevicomis* were captured throughout the nine trapping locations in Arizona. In seven out of nine locations, the most effective lure for trapping these two species was the combination of *exo*-brevicomin, frontalin, and α-pinene (stats). In the other two locations, myrcene in combination with *exo*-brevicomin and frontalin was the most attractive. At the Haulapai Mountains location (west side of State), only *D. brevicomis* was captured. Due to large capture numbers and poor conditions of the insects in many traps, not all *Dendroctonus* could be identified as *frontalis* or *brevicomis*. Of those insects that could be identified to both species and sex, ... Sex ratio...and lure preference?

Relatively low numbers of predators, compared to Experiment 1, were captured in our traps (Table 4). Of the predators captured, most species were attracted to the WPB lure + terpene blend or α-pinene over the WPB lure with myrcene.

4 Discussion

The standard commercial lures for attracting *D. brevicomis* and *D. frontalis* were not as effective at capturing beetles and beetle-predators as other pheromone-terpene combinations tested. In contrast to studies in other locations (Vite and Renwick 1971, Payne et al. 1977, Bedard et al. 1969, 1980), the lure comprised of *exo*-brevicomin + frontalin + α-pinene was significantly most attractive to both *D. frontalis* and *D. brevicomis*. The presence of *exo*-brevicomin and particular terpenes were important variables in determining trap catches of *Dendroctonus* and their predators in Arizona.

The increased attraction by *D. frontalis* and *D. brevicomis* to lures with α-pinene over lures with myrcene suggests that the monoterpene component can significantly affect the attraction and arrestment behavior of these species. The ternary mixtures of *exo*-brevicomin, frontalin and a terpene were consistently the most attractive to both of these *Dendroctonus* species. Regional differences in attraction of *D. brevicomis* to particular terpenes have been reported several times. For instance, Bedard et al. (1969) and Pitman and Vite (1971) reported that myrcene in combination with *exo*-brevicomin and frontalin was more attractive to *D. brevicomis* than other terpene compounds in California, while Bedard et al. (1980) later found no difference in attraction containing various monoterpenes to *D. brevicomis*. Differences in *D. brevicomis* attraction to pheromone combinations reported in these studies may arise from (1) temporal and spatial differences in beetle attraction to specific terpenes, (2) differences in experimental methodology, or (3) differences in stereochemistry, release rates, and purity of test compounds. If differences in attraction to lure combinations are representative of what is happening in nature, then the relative effectiveness of commercially available lures likely changes with geographic location. The high attraction of *D. frontalis* to α-pinene appears universal (Renwick and Vite 1969), and increased release rates of α-pinene increases beetle attraction. However, inclusion of α-pinene in a lure did not always make it the most attractive in our study. For *D. frontalis* and *D. brevicomis* in Arizona, in two out of nine locations the combination of *exo*-brevicomis, frontalin and α-pinene was not as attractive as the combination with the myrcene.

The finding that *D. frontalis* is attracted to *exo*-brevicomin, in combination with frontalin and a monoterpene, is contrary to earlier studies of the beetle in the southeast United States where *D. brevicomis* does not occur (Payne et al. 1977, Richerson and

Payne 1979, Watterson et al. 1982). Payne et al. (1977) found that *exo*-brevicomin reduced or had no affect on *D. frontalis* landing rates within infestations in Texas. However, recent studies by Hofstetter et al. (in press) in Mississippi found that the addition of *exo*-brevicomin to the SPB lure significantly increased trap captures. Our findings are consistent with previous findings in Arizona that demonstrated attraction of *D. frontalis* to *exo*-brevicomin (Sanchez-Martinez & Wagner 2002, Zausen et al. 2005, Gaylord et al. 2006). This may not be surprising in that *D. frontalis* antenna responses to *exo*-brevicomin and frontalin have similar thresholds and sensitivities (Payne 1975, Pureswaran et al., in press).

Attraction of *D. frontalis* to *exo*-brevicomin may depend upon the release rate of *exo*-brevicomin, the proximity of the lure to attacked trees, or density of beetles within the area. For instance, outside of infestations *D. frontalis* may be attracted to *exo*-brevicomin (Hofstetter et al., in press) but inside infestations *exo*-brevicomin may be disruptive (Payne et al. 1977). Another explanation is that *D. frontalis* is attracted to impurities associated with our *exo*-brevicomin lure. *Endo*-brevicomin, which is a significant component of male *D. frontalis* pheromone in the southeast U.S. (Pitman et al. 1969, Sullivan et al. 2007), may be attractive to *D. frontalis* in the field. However, the quantity of *endo*-brevicomin in the WPB lure is minute, less than 1% of that of *exo*-brevicomin, and is unlikely to significantly attract beetles (Hofstetter et al., in press). Another explanation is that *D. frontalis* is equally attracted to either stereochemistry of brevicomin, at least outside of beetle infestations as reported here and by Hofstetter et al. (in press).

The attraction of both *Dendroctonus* species to *exo*-brevicomin suggests that *D. frontalis* and *D. brevicomis* may utilize this compound in conjunction to mass aggregate and overcome host tree defenses. Both species are observed to colonize ponderosa pine in Arizona and co-occur within the same tree (Breece et al., in review). This is contrary to the belief that *D. frontalis* and *D. brevicomis* displace or exclude each other from habitats or areas (Lanier et al. 1988). The advantage each species gains in having cross attraction of pheromones to mass attack trees (Reid 1999, Ayres et al. 2001) may counterbalance selection for reproductive isolation and the negative effects of interspecific competition (Symonds & Elgar 2004). Mate recognition and reproductive

isolation between these species may result from differences in their pheromone composition that is not represented in the commercial lures (Byres 1989) or from differences in short-range acoustic communication once beetles have landed on the tree (Rudinsky 1973, Rudinsky et al. 1974). Both species have common pheromone components (frontalin, *endo*-brevicomin, *trans*-verbenol, and verbenone; Hofstetter et al. in press) so one would expect that the pheromone that occurs in one species but not in the other (i.e. *exo*-brevicomin not produced by *D. frontalis*) would mediate species specificity in pheromone communication, by being repellent, and thereby maintain reproductive isolation between the two species when they occur in sympatry. Comparison of acoustic specificity in sympatric and allopatric bark beetles may show reproductive character displacement not found in olfactory signals (Symonds & Elgar 2004). Behavioral mechanisms for avoiding interspecific competition likely occur between these species, but are yet unknown.

Predator responses to terpene-beetle pheromone combinations varied with the predator species. The *T. chlorodia* kairomone response appears rather specialized for *D. brevicomis* (Bedard et al. 1969, Pitman and Vite 1971), in that it responds strongly to *exo*-brevicomin and less so to frontalin. *exo*-Brevicomin is released by other *Dendroctonus* species that occur in Arizona and *T. chlorodia* may use this compound to successfully locate a diverse prey base. For *D. frontalis*, the co-attraction to *D. brevicomis* pheromones (i.e. *exo*-brevicomin) to successfully overcome tree defenses may outweigh potential losses due to predation by *T. chlorodia* or interspecific competition for space by beetle larvae. The beetle *Elacatis* was strongly influenced by the terpene component, with lure combinations containing α-pinene being most attractive. The biology and predatory behavior of *Elacatis* is not well known but is suspected to be a generalist predator of both primary and secondary bark beetles (Gaylord et al. 2006). Its attraction to volatile cues from host trees under beetle attack may allow it to locate a variety of prey. Too few *Enoclerus* spp. were caught during our experiments to make statistical inferences on kairomonal responses. However, previous studies have shown attraction to lures containing ipsdienol (Miller and Borden 1990) and *trans*-verbenol (Schmitz 1978).

Acknowledgements

We thank Elisabeth Alden, Gabe DeGomez, Kevin Johnson, Laine Smith, and James Stewart for assistance in collecting and counting insects. We also thank Fool Hollow State Park, Apache/Sitgreaves NF, Coronado NF, Coconino NF, Prescott NF, and Tonto NF for trap collections. We thank two anonymous reviewers. This project was funded by Western Bark Beetle Initiative U.S.D.A. 05-PA-11221615-161, U.S.D.A. Cooperative Agreement 06-CA-11330129-046, and supported by School of Forestry Northern Arizona University and U.S.D.A. Forest Service.

References

Aukema, B.H., K.F. Raffa. 2005. Selective manipulation of predators using pheromones: responses to frontalin and ipsdienol pheromone components of bark beetles in the Great Lakes region. Agric. Forest Entomol. **7,** 193–200.

Ayres, B.D., M.P. Ayres, M.D. Abrahamson and S.A. Teale. 2001. Resource partitioning and overlap in three sympatric species of *Ips* bark beetles (Coleoptera: Scolytidae). Oecologia **128,** 443–453.

Bedard, W.D., P.E. Tilden, D.L. Wood, R.M. Silverstein, R.G. Brownlee, and J.O. Rodin. 1969. Western pine beetle: Field response to its sex pheromone and a synergistic host terpene, myrcene. Science 164: 1284-1285.

Bedard, W.D., D.L. Wood, P.E. Tilden, K.Q. Lindahl Jr., R.M. Silverstein and J.O. Rodin. 1980. Field response of the western pine beetle and one of its predators to host- and beetle-produced compounds. J. Chem. Ecol. 6: 625-641.

Billings, R.F. and C. M. Bryant. 1983. Developing a system for mapping the abundance and distribution of southern pine beetle habitats in east Texas. Z. Angew. Entomol. 96: 208-216.

Breece, C. R., T. E. Kolb, B. G. Dickson, J. D. McMillin and K. M. Clancy. Prescribed fire effects on bark beetle activity and tree mortality in southwestern ponderosa pine forests. Forest Ecol. Manag. In review.

Byers, J.A. 1989. Behavioral mechanisms involved in reducing competition in bark beetles. Holartic Ecology 12: 466-476.

Byers, J.A. 1995. *In*... (Editors...). Host-tree chemistry affecting colonization in bark beetles. XXX. Pages 154-213.

Carmer, S.G. and M.R. Swanson. 1973. Evaluation of ten pairwise multiple comparison procedures by Monte Carlo methods. J. American Statistical Association 68: 66-74.

Feeny, S.R., T.E. Kolb, W.W. Covington, and M.R. Wagner. 1998. Influence of thinning and burning restoration treatments on presettlement ponderosa pines at the Gus Pearson Natural Area. Can. J. Forest Res. 28: 1295-1306.

Gaylord, M.G., T.E. Kolb, K.F. Wallin, and M.R. Wagner. 2006. Seasonality and Lure Preference of Bark Beetles (Curculionidae: Scolytinae) and Associates in a Northern Arizona Ponderosa Pine Forest. Environ. Ent. 35 (1): 37-47.

Herms et al. 1991

Hofstetter, R.W., D.S. Pureswaran, and B.T. Sullivan. 2007. Attraction of *exo*-brevicomin to *Dendroctonus frontalis* (Coleoptera: Curculionidae: Scolytinae) in the southeast United States. Agric. Forest Entomology (in prep).

Hopkins, A.D. 1909. Contributions toward a monograph of scolytid beetles. I. The genus *Dendroctonus*. U.S. Dep. Agric. Bur. Entomol. Tech. Serv. 17.

Kolb, T.E., K.M. Holmberg, M.R. Wagner, and J.E. Stone. 1998. Regulation of ponderosa pine foliar physiology and insect resistance mechanisms by basal area treatments. Tree Physiol. 18: 375-381.

Lanier, G.N., J.P. Hendrichs & J.E. Flores. 1988. Biosystematics of the *Dendroctonus frontalis* (Coleoptera: Scolytidae) complex. Annals of the Entomological Society of America 81(3): 403-418.

Lindgren, B. S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Canadian Entomologist. 115:299-302.

Namkoong, G., J.H. Roberds, L.B. Nunnally, and H.A. Thomas. 1979. Isozyme variation in populations of southern pine beetle. For. Sci. 25: 197-203.

Moser, J.C., H. Konrad, T. Kiristis and L. K. Carta. 2005. Phoretic mites and nematode associates of *Scolytus multistriatus* and *Scolytus pygmaeus* (Coleoptera: Scolytidae) in Austria. Agric. Forest Entomol. 7: 169-177.

Payne, T.L. 1975. Bark beetle olfaction. III. Antennal olfactory responsiveness of *Dendroctonus frontalis* Zimmerman and *D. brevicomis* Le Conte (Coleoptera: Scolytidae) to aggregation pheromones and host tree terpene hydrocarbons. J. Chem. Ecol. 1: 233-242.

Payne, T.L., J.E. Coster and P.C. Johnson. 1977. Effects of slow-release formulation of synthetic *endo*- and *exo*-brevicomin on southern pine beetle flight and landing behavior. J. Chem. Ecol. 3: 133-144.

Pitman, G. B., J. P. Vité, G. W. Kinzer, and A. F. F. Jr. 1969. Specificity of population-aggregating pheromones in *Dendroctonus*. J. Insect Physiol. 15:363-366.

Pitman, G.B. and J.P. Vite. 1971. Predator-prey response to western pine beetle attractants. J. Econ. Entomol. 3: 886-887.

Poland, T.M. and J.H. Borden. 1997. Attraction of a bark beetle predator, *Thanasimus undatulus* (Coleoptera: Cleridae), to pheromones of the spruce beetle and two secondary bark beetles (Coleoptera: Scolytidae). J. Entomol. Soc. Brit. Columbia 94: 35-41.

Poland, T.M. and J.H. Borden. 1998. Disruption of secondary attraction of the spruce beetle, *Dendroctonus rufipennis*, by pheromones of two sympatric species. J. Chem. Ecol. 24: 151-166.

Raffa, K. F. 2001. Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. Chemoecology 11:49-65.

Raffa KF Dahlsten DL, 1995. Differential responses among natural enemies and prey to bark beetle pheromones. Oecologia. **102,**17–23.

Reid, M.L. 1999. Monogamy in the bark beetle Ips latedens: ecological correlates of an unusual mating system. Ecol. Entomol. 24: 89-94.

Renwick, J.A.A. and J.P. Vite. 1969. Bark beetle attractants: mechanisms of colonization by *Dendroctonus frontalis*. Nature 244: 1222-1223.

Richerson, J.V. and T.L. Payne. 1979. Effects of bark beetle inhibitors on landing and attack behavior of the southern pine beetle and beetle associates. Environ. Entomol. 8: 360-364.

Rudinsky, J.A. 1973. Multiple functions of the southern pine beetle pheromone verbenone. Environ. Entomol. 2: 511-514.

Rudinsky, J.A., M.E. Morgan, L. M. Libbey and T.B. Putman. 1974. Anti-aggregation – rivalry pheromone of the mountain pine beetle and new arrestant of the southern pine beetle. Environ. Entomol. 3: 90-98.

Sanchez-Martinez, G. and M.J. Wagner. 2002. Bark beetle community structure under four ponderosa pine forest stand conditions in northern Arizona. Forest Ecol. Manag. 170: 145-160.

Smith, R.H. 2000. Xylem monoterpenes of pines: Distribution, variation, genetics, function. Gen. Tech. Report PSW-GTR-177. 454 pages.

Sturgeon, K.B. 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. Evolution 33: 803-814.

Symonds, M.R.E. and M.A. Elgar. 2004. Species overlap, speciation and the evolution of aggregation pheromones in bark beetles. Ecology Letters 7: 202-212.

Sullivan, B. T., W. P. Shepherd, D. S. Pureswaran, and K. Mori. 2007. Evidence that (+)-*endo*-brevicomin is a male produced aggregation pheromone component of the southern pine beetle, *Dendroctonus frontalis*. Journal of Chemical Ecology submitted

Vite, J. P., and J. A. A. Renwick. 1971. Inhibition of *Dendroctonus frontalis* response to frontalin by isomers of brevicomin. Naturwissenschaften 58:418.

Watterson, G.P., T.L. Payne and J.J. Richerson. 1982. The effects of verbenone and *endo*-brevicomin on the within tree populations of *Dendroctonus frontalis*. J. Geo. Entomol. Soc. 17: 118-126.

Wood, D.L. 1982a. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Ann. Rev. Entomol. 27: 411-446.

Wood, S.L. 1982b. The Bark and Ambrosia Beetles of North and Central America (Coleoptera: Scolytidae), a Taxonomic Monograph. Great Basin Naturalist Memoirs No. 6, Brigham Young University, Provo, Utah.

Zausen, G.L., T.E. Kolb, J.D. Bailey and M.R. Wagner. 2005. Long-term impacts of stand management on ponderosa pine physiology and bark beetle abundance in northern Arizona: A replicated landscape study. For. Ecol. Manag. 218: 291-305.

Zhou J Ross DW Niwa CG, 2001. Kairomonal response of *Thanasimus undatulus*, *Enoclerus sphegus* (Coleoptera: Cleridae), and *Temnochila chlorodia* (Coleoptera: Trogositidae) to bark beetle semiochemicals in eastern Oregon. Environ. Entomol. **30,** 993–998.

Table 1. Chemical components of terpene blend used in lures during Experiment 2, and monoterpene composition of resin from 64 ponderosa pine in the Coconino N.F., Arizona compared to resin composition of ponderosa in Sierra Nevada California. Terpene blend created by ChemTica. Arizona monoterpene analyses by J. Mahfouz, USDA Forest Service, Southern Research Station (unpublished).

Terpenes	Terpene Blend Lure (% comp.)	Arizona (% mean ± STD)	California (%)*
α-pinene	45	46 ± 15	1
β-pinene	15	7 ± 13	50
3-carene	24	31 ± 13	30
Myrcene	12	3 ± 2	3
Terpinolene	4	2 ± 1	?
Limonene	0	7 ± 6	10
β-cubebene	0	3 ± 3	?
Borneol acetate	0	1 ± 1	0
others			6
	100	100	100

* Resin composition of ponderosa pine from Sierra Nevada near Placerville, CA. (Smith 1975)

Table 2. Total numbers of insects collected from Lindgren funnel traps in Arizona during the two trapping experiments.

Insect	Family	Guild	Exp. 1 Totals	Exp. 2 Totals
Dendroctonus adjunctus	Scolytinae	Herbivores	57	0
D. approximatus	(Curculionidae)		60	8
D. brevicomis			11032	9362
D. frontalis			15241	4012
D. valens			290	6
Hylastes spp.			15	1
Ips spp.			145	1
Xyleborus spp.			6	3
Scolytus spp.			3	0
Metallic woodborers	Buprestidae	Herbivores/Predators	21	3
Longhorn beetles	Cerambycidae		96	13
Elacatis sp.	Othniidae	Predators	189	22
Enoclerus spp.	Cleridae		81	7
Platysoma spp.	Histeridae		18	1
Temnochila spp.	Trogositidae		683	138
Tenebroides spp.	Trogositidae		59	13
Click beetle spp.	Elateridae	Unknown	278	5

Table 3. F-values for mixed model ANOVA for the most abundant insects collected in Lindgren funnel traps during Experiment 1. ** indicate p-value <0.01

		D. brevicomis	***D. frontalis***	***D. valens***	***Elacatis sp.***	***T. chlorodia***	**Elaterid**
Effect	*DF*						
Week	5	3.97**	0.54	9.31**	6.81**	17.45**	4.97**
Treatment	6	246.8**	142.9**	4.69**	7.00**	24.03**	0.90
Trtmt*Week	30	2.17**	2.68**	1.28	1.35	2.20**	0.92

Table 4. Trap catch data for Experiment 2…

Location	Site #	Sub-sites	Total *D. frontalis* & *D. brevicomis*	Percent *D. frontalis*	Proportion of *Dendroctonus* spp. ca	
					WPB w/myrcene	WPB w/pinene
Follow Hollow Lake	1	1	1271	20	0.0	0.58
Black Mesa R.D.	2	1	1001	73	0.16	0.43
Cent. Forest	3	1	84	39	0.46	0.36
Kendrick Mt.	4	1				
Morman Lake	5	1	249	66	0.67	0.12
Tonto N.F.	6	1	3221	17	0.17	0.68
		2	1343	19	0.27	0.42
Prescott N.F.	7	1	1253	10	0.14	0.52
		2	3221	10	0.17	0.51
Haulapai Mts.	8	1	828	0	0.24	0.41
			13374	**27**	**0.25**	**0.45**

Table 5. F-values for mixed model ANOVA for the most abundant insects collected in traps during Experiment 2. * indicate p-value <0.05; ** indicate p-value <0.01

		Total bark beetles	***D. brevicomis***	***D. frontalis***			
Effect	*DF*						
Location	8						
Treatment	2						
Trtmt*Local	16						

For submission to:
Agriculture and Forest Entomology

Differences in attraction of primary and secondary bark beetles and their predators to terpene levels and pheromone combinations in Arizona

R.W. HOFSTETTER* [1], S. MARTINSON[2], M.L. GAYLORD[1] and M. WAGNER[1]

[1] School of Forestry, Northern Arizona University, Flagstaff AZ 86011 USA
[2] Department of Biological Sciences, Dartmouth College, Hanover NH 03755 USA

* Corresponding author. Mailing address: School of Forestry, Northern Arizona University, Flagstaff, AZ 86011-5018. Phone: (928) 523-6452. FAX: (928) 523-1080.
Rich.Hofstetter@nau.edu

Introduction

Bark beetles that exploit dead and living trees are generally divided into two life history strategies, classified as 'primary' and 'secondary' bark beetles (Wood 1982). Primary bark beetles are obligate parasites of living trees and kill trees as a result of mass colonization. These beetles are considered obligate parasites in that their offspring do not survive if oviposited into dead trees. Secondary bark beetles are facultative parasites capable of colonizing weakened, stressed and recently killed trees. These beetles are facultative in that their offspring are capable of survival in trees that are dead or living prior to oviposition. At low population densities, secondary bark beetles will colonize trees stressed by disease, drought, pathogens, and crowding, or attacked by primary bark beetles. During high population densities, secondary bark beetles can colonize and kill healthy trees (Paine et al. 1997) or simultaneously infest trees colonized by other beetle species, resulting in interspecific competition for food and space (Ayres et al. 2001, Hedgren 2004, Poland & Borden 1998).

Pre-attack olfactory mechanisms for avoiding competition would be mutually advantageous to species competing for the same resource. Competitive displacement via beetle specific pheromones has been observed between secondary and primary bark beetles in several systems: *I. pini* and *D. ponderosae* Hopkins in lodgepole pine in northwest United States (Rankin & Borden 1991, Safranyik et al. 1996), *D. rufipennis* and *I. tridens* in spruce in British Columbia, Canada (Poland & Borden 1998), and *I. paraconfusus* and *D. brevicomis* in ponderosa pine in California (Byers & Wood 1980). Alternatively, the colonization of healthy trees requires hundreds to thousands of beetles, and cooperation among species may increase the

chance of successful colonization of host trees. In this case, multiple species may have positive taxis toward pheromones emitted by others colonizing species. For instance, secondary bark beetle species may find weakened and susceptible host trees by orienting to volatiles produced by competing insect species during colonization (Byers 1995). For example, *D. rufipennis* lures enhanced attraction of the secondary bark beetle *Dryocoetes affaber* to its pheromone (Poland & Borden 1998), and *I. paraconfusus* responded to pheromone components of *Dendroctonus* species (Byers & Wood 1981).

Volatiles released from attacked host trees, in combination with aggregation pheromones, can influence bark beetle attraction and alter beetle preferences to pheromones of potentially interfering bark beetles species (Byers 1995). The presence of most tree volatiles may indicate that the host tree is alive and that the attack phase is not complete, and thus may enhance attraction of primary bark beetles species (i.e. *D. frontalis* and *D. brevicomis*). For secondary bark beetles such as *I. pini* that have aggregation pheromones but are facultatively aggressive, host tree monoterpenes may have minimal or negative effects on *Ips* attraction. Alternatively, secondary species that do not use aggregation pheromones may have a strong attraction to host monoterpenes (Schroeder & Lindelow 1989).

Interspecific communication among species is not limited to bark beetles. Bark beetle predators specifically exploit beetle pheromones (as kairomones) to locate prey. Predator species may be species-specific in their preference of pheromones or be regionally specific to particular pheromone compositions ( ). Additionally, predators may be more or less attracted to volatiles from host trees, depending on the host selection behavior of their prey (i.e., living vs dead host trees). Predators of secondary versus primary bark beetles may use different suites of chemical cues to locate their prey. For predators of secondary bark beetles, bark beetle pheromones probably play a greater role in location of prey than host tree volatiles. While predator specialists of primary bark beetles likely use a combination of beetle pheromone and host volatiles to find their prey.

Our objectives are to determine (1) the effects of host volatiles on the attraction of primary and secondary bark beetles to their pheromone and the pheromone of conspecifics, (2) whether males and females of secondary and primary bark beetle species are differentially affected by the presence host volatiles, and (3) the effects of host volatiles and kairomone combinations on bark beetle predators. We focused on four abundant bark beetles species (the pine engraver *Ips pini* Say, the six-spined pine engraver *Ips calligraphus* sub. *ponderosae* (Swaine), the southern pine beetle *Dendroctonus frontalis* Zimmerman, and the western pine beetle *Dendroctonus brevicomis* LeConte) and their predators in Ponderosa pine forests of central Arizona, United States. These bark beetle species are believed to have sustained low populations for hundreds of years in Arizona without reaching outbreak levels. Interactions between these species likely occur as many are seen to cohabit the same trees (references). Whether these species are attracted or displaced by aggregation pheromones of conspecifics (synomones) in this region is not known. *Ips pini* and *I. calligraphus* are secondary bark beetles that usually infests downed trees, logging slash or the tops of weakened trees, but at high densities will infested standing, healthy trees (Livingston 1979, Kolb et al. 2006). *Dendroctonus frontalis* and *D. brevicomis* are primary bark beetles that attack the bole of living pines (Wood 1982), resulting in tree mortality. These four species often colonize the same host tree, with the *Dendroctonus* species colonizing the bole of the tree, *I. calligraphus* attacking the bole and large branches, and *I. pini* often colonizing the upper portion of the tree bole. However, all four species can co-occur in the same area of the bole. The species may benefit each other by

procuring the host tree, but compete for resources and interfere with mating once the tree's defenses are overcome.

We tested four hypotheses. Hypothesis 1: Secondary bark beetles (e.g., *Ips pini*) are attracted to pheromones of primary bark beetles (*Dendroctonus* spp.). Hypothesis 2: Pheromones from secondary bark beetles disrupt communication among primary bark beetles. The presence of pheromones from secondary bark beetles may signify that the tree is no longer suitable for colonization by primary bark beetles, and the presence of pheromones of secondary bark beetles disrupts attraction of primary bark beetles to their own pheromone. Hypothesis 3: The combination of monoterpenes and pheromones increases attraction to primary bark beetles but decreases attraction to secondary bark beetles. Monoterpenes are attractive to a large number of bark beetles and synergism between bark beetle pheromones and various monoterpenes is widespread (Scriber 1984, Byers). The presence of monoterpene volatiles may signify that the tree still contains resin that may impede colonization by secondary bark beetles. For primary bark beetles, the present of monoterpenes may be a prerequisite for attack. Additionally, primary bark beetles have a greater ability to survive the toxic monoterpenes within the bark and phloem of living trees than secondary bark beetles (Hodges et al. 1979). Hypothesis 4: The addition of monoterpenes to bark beetle pheromones will alter the predator composition. Many parasites and predators of bark beetles are strongly attracted to bark beetle pheromones (Poland & Borden 1997, Aukema & Raffa 2005), but the attraction of predators to particular beetle pheromones is influenced by the presence of terpenes (Pitman & Vite 1971).

Material and methods

The trapping experiment was conducted in natural Ponderosa pine (*Pinus ponderosae* Douglas ex. Lawson) stands within the Coconino National Forest of central and northern Arizona. Ten blocks of eight 12-unit funnel traps (Lindgren 1983) were arranged in linear arrays, with 50 m between traps. Blocks were spaced a minimum of 1 km apart. Each trap was hung on metal conduit at least 3 m from the nearest tree. The bottoms of the traps were 1 m above the ground. Each trap was randomly allocated to a treatment and re-randomized each week. Lures were attached to the middle funnel of the trap and a 3x3 cm section of No-pest Strip ( ) was placed into each collection cup to kill captured insects and reduce predation by predatory insects. Trap catches were collected every 3 to 4 days from July 28, 2005 to September 6, 2005. All insects were identified to species or genus, except for large wood borers and click beetles which were identified to family. Males and females were counted for *Ips pini*, I. calligraphus, *Dendroctonus frontalis*, and *D. brevicomis*.

The following eight treatments were tested: 1) blank control (no lure), 2) Ips pini lure (-97/+3 ipsdienol and lanierone; Phero Tech Inc., Delta, British Columbia), 3) Ips pini lure + low release of α-pinene, 4) Ips pini + high release of α-pinene, 5) Western pine beetle lure (exo-brevicomin + frontalin + myrcene; Phero Tech Inc., Delta, British Columbia), 6) Western pine beetle lure with myrcene replaced by low α-pinene, 7) Western pine beetle lure with myrcene replaced by high release α-pinene, 8) Ips pini lure + Western pine beetle lure with myrcene replaced by high release α-pinene. The frontalin was dispensed from 400*ul* polyethylene bottles with a release rate of 2.0 ± 0.2 mg/day under field conditions, myrcene was dispensed from 1.8ml polyethylene bottles with a release rate of 1.0 ± 0.1 mg/day under field conditions, and *exo*-brevicomin were dispensed from ...at a release rate of... mg/d in the field during our study.

The α-pinene (99% pure, SigmaAlrich) components were created by S. Martinson and had release rates of 2.2 ± 0.3 mg/day and 21.7 ± 0.5 mg/day for the low and high doses, respectively.

Analysis of variance was performed on each insect species for which substantial numbers (> 5 individuals per trapping period) were captured. Data were analyzed using a split plot design (SAS PROC MIXED; SAS *year*). Block and Block by Treatment interaction were considered random effects. In some cases, we tested combinations of predator or competitor species that showed significant treatment effects. Data were square-root transformed before analysis to reduce heteroscedasticity, although graphs and figures show raw means and standard errors. Where significant treatment effects occurred ($\alpha = 0.05$), differences were separated by pairwise *t*-tests on least squared means (Carmer & Swanson, 1973).

Results

A total of 11,875 insects over a 39-day period were captured representing more than 22 insect species (Table 1). Bark beetles and bark beetle predators accounted for 67% and 29% of the insects collected, respectively. The remaining 4% of insects consisted of large wood borers, click beetles and other bark inhabiting insects. The most abundance bark beetle species was *Ips calligraphus* followed by *Dendroctonus frontalis*, *Ips pini*, and *D. brevicomis*. Low numbers of *Hylastes* sp., *D. valens*, *D. approximatus*, *I. lecontei*, *D. adjunctus*, and *I. latedens* were also collected. The most abundant bark beetle predator was *Temnochila chlorodia* (Coleoptera: Trogositidae) followed by *Elacatis* sp. (Coleoptera: Othniidae) and *Enoclerus lecontei* (Coleoptera: Cleridae). Other predators captured, but in low numbers, included several *Tenebroides* and *Platysoma* spp. Trap catches of__ insects were relatively uniform throughout the experiment while all other abundant species varied considerably from week to week (Table 2). There was a significant week x lure interaction for several herbivore and predator species (Table 3).

Primary bark beetles:

The two prevalent primary bark beetles in these forests, *Dendroctonus brevicomis* and *D. frontalis* showed a significant attraction to the western pine beetle lure, but differed slightly in their responses to pheromones of secondary bark beetles and to the terpene component and terpene release rate (Fig. 1a & b). *D. frontalis* was very weakly attracted to the Ips pini lures and the combination of the Ips pini lure and the western pini lure (with high release rate of a-pinene) reduced traps catches slightly, equaling that of the western pine beetle lure with a low α-pinene release rate. The addition of the Ips pini lure to the western pine beetle lure did not negatively affect the attraction of *D. brevicomis*. The Ips pini lure, without α-pinene, was slightly attractive to *D. brevicomis*. In general, the replacement of the myrcene component of the western pine beetle lure with α-pinene attracted >2 times as many beetles. *Dendroctonus frontalis* attraction increased by 2x and 6x to the western pine beetle lure with the low and high release α-pinene lures, respectively, while *D. brevicomis* attraction only increased with the low α-pinene release rate lure. Overall, 86% and 44% of *D. frontalis* and *D. brevicomis*, respectively, were males. For both species, significantly more males than females were captured in the traps with the lure combination: Ips pini lure + western pine beetle lure with the high release of a-pinene.

Although very few *D. adjunctus*, *D. approximatus* and *D. valens* were captured, they showed preferences for particular lure combinations. *D. adjunctus* was only attracted to the western pine beetle lure, with most catches occurring in traps with the low release rate of α-

pinene. *D. approximatus* was only attracted to the western pine beetle lure and trap catches increased with increased release of α-pinene. *D. valens* were captured in traps with the Ips pini lure and/or the western pine beetle lure, and trap captures increased with increased release of α-pinene. For both *D. valens* and *D. approximatus*, the combination of the Ips pini lure and the western pine beetle lure with the high release rate of α-pinene attracted the greatest number of beetles. Sex ratios were not determined for these species.

Secondary bark beetles:

The two most abundant secondary bark beetles, *Ips calligraphus* and *I. pini*, showed a significant attraction to the Ips pini lure, but differed in their responses to the primary bark beetle lure and the terpene component and terpene release rate (Figs 2a & b).. *Ips calligraphus* was most attracted to the combination of Ips pini and the western pine beetle lure with the high release α-pinene. The western pine beetle lure with myrcene or the low release α-pinene was not attractive to the beetle. *Ips pini* showed no attraction to the western pine beetle lure without the addition of the Ips pini lure component. The addition of α-pinene to the Ips pini lure increased catches of *Ips calligraphus* but decreased catches of *Ips pini*. Increased release rate of α-pinene resulted in greater *I. calligraphus* but lower *I. pini* catches. Male: female ratios were 1:1.2 for both *Ips* species and did not change with lure treatments. *Ips latedens* and *I. lecontei* were captured but there were too few to draw conclusions on lure preferences. The secondary bark beetles *Hylastes* (spp.) were collected and were significantly attracted to the western pine beetle lures with α-pinene, including the western pine beetle lure with the Ips pini lure.

Bark beetle predators:

Temnochila chlorodia was the most abundant bark beetle predator (Table 1) and was most attracted to lures containing the high release rate α-pinene (Fig. 3A). Overall, all three prominent predators (*Temnochila*, *Elacatus*, and *Enoclerus* spp.) were more attracted to the Ips pini lure than the Western pine beetle lure (Fig. 3), and the combination of the Ips pini lure and Western pine beetle lure with the high release rate α-pinene was most attractive to these predators. The blank control and the western pine beetle lure (without α -pinene) were not significantly different, suggesting that the α-pinene component and not the western pine beetle lure, is an important attractant for these predators. Other predators in the families Histeridae and Trogostidae and large wood borers in the families Cerambycidae and Buprestidae were collected too infrequently to draw conclusions.

Acknowledgements

We thank Elizabeth Alden, Laine Smith, James Steward, Gabe DeGomez, Greg Zausen. Discussion with Joel McMillin, Tom Degomez for help in the field and project ideas. This project was funded by Western Bark Beetle Initiative U.S.D.A. 05-PA-11221615-161, U.S.D.A. Cooperative Agreement 06-CA-11330129-046, Dartmouth College Kramer fund, and supported by School of Forestry Northern Arizona University and U.S.D.A. Forest Service.

Table 1. Total numbers of beetles collected from trapping study during Summer 2005 in the Coconino National Forest, Arizona.

Insect	Family	Guild	Total
Dendroctonus adjunctus	Scolytidae	Herbivores	36
D. approximatus	(Curculionidae)		92
D. brevicomis			502
D. frontalis			2489
D. valens			105
Hylastes spp.			123
Ips calligraphus			2248
Ips latedens			15
Ips lecontei			54
Ips pini			2323
Metallic woodborers	Buprestidae	Herbivores/Predators	20
Longhorn beetles	Cerambycidae		211
Cossonus sp.	Curculionidae		4
Corticeus sp.			3
Click beetle spp.	Elateridae	Unknown	174
Elacatis sp.	Othniidae	Predators	872
Enoclerus lecontei[1]	Cleridae		822
Enoclerus sp. [2]			15
Platysoma spp.	Histeridae		52
Temnochila chlorodia	Trogositidae		1677
Tenebroides sp.	Trogositidae		38
		Total:	**11,875**

[1] Several *Enoclerus lecontei* were *E. sphegeus*.
[2] *Enoclerus* sp. has a red abdomen and is much larger than *E. lecontei* or *E. sphegeus*.

Environmental Entomology
Community and Ecosystem Ecology

M. L. Gaylord
School of Forestry
Northern Arizona University
PO Box 15108
Flagstaff, AZ 86011
Phone: 928 523 9200
Fax : 928 523 1080
E-mail: mlg36@ nau.edu

Influence of temperature on spring flight initiation for Southwestern ponderosa pine bark beetles (Coleoptera: Curculionidae, Scolytinae)

M. L. Gaylord[1], K. B. Williams[2], R. W. Hofstetter[1], J. D. McMillin[3], T. E. DeGomez[2], and M. R. Wagner[1]

1. School of Forestry, Northern Arizona University
P.O. Box 15018, Flagstaff, AZ 86011

2. University of Arizona, School of Natural Resources, NAU Box 15018, Flagstaff, AZ 86011

3. USDA Forest Service, Southwestern Region, Forestry and Forest Health, Arizona Zone, 2500 S. Pine Knoll Drive, Flagstaff, AZ 86011-6381

ABSTRACT

Determination of flight initiation and activity is a cornerstone of pest management for many economically important insects. Knowledge of this information for bark beetles (Coleoptera: Curculionidae, Scolytinae) can facilitate timing of management strategies, such as single tree protection treatments. Our goals were to determine optimal temperature predictors for bark beetle flight of three species of *Ips* bark beetles, five species of *Dendroctonus* beetles and two genera of bark beetle predators, *Enoclerus spp.* (Coleoptera: Cleridae)and *Temnochila chlorodia* (Mannerheim) (Coleoptera: Ostomidae), that occur in the ponderosa pine forests of northern Arizona. We quantified beetle flight activity using data loggers and pheromone-baited Lindgren funnel traps at 19 sites over four years. Ambient air temperature was monitored using temperature data loggers located in close proximity to funnel traps. We analyzed degree day accumulation and differences between minimum, average and maximum ambient temperature for the week prior to and week of first beetle capture to calculate flight temperature thresholds. Degree day accumulation was inconclusive and not a good predictor for initiation of beetle flight. For all species analyzed other than *D. adjunctus*, spring time temperatures needed to exceed 15 °C before beetles were captured in traps. However, once initial flights had begun, beetles were often captured when maximum ambient air temperatures were below this threshold temperature. Maximum and average air temperatures were a better predictor for beetle flight initiation than minimum temperature. We discuss our results in the context of management implications and global climate change.

Keywords: Arizona, Degree day, *Dendroctonus*, *Enoclerus,* Flight, *Ips*, *Temnochila,* temperature, ponderosa pine,

INTRODUCTION

Determination of flight initiation and activity is a cornerstone of pest management for many economically important insects. Temperature is generally acknowledged as a key factor regulating seasonality of insects, including bark beetles (Coleoptera: Curculionidae, Scolytinae) (Zaslavski 1988, Powell et al. 2000) by directly impacting development rates (Miller and Keen 1960, Hansen et al. 2001b, Netherer and Pennerstorfer 2001). This information can be used to develop degree day models to predict the onset of bark beetle activity (Pruess 1983, Higley et al. 1986, Ayres et al. 2001, Hansen et al. 2001, Kennedy and McCullough 2002) and temperature thresholds required for flight (e.g. Miller and Keen 1960, McCambridge 1971, Livingston 1979, Holsten and Hard 2002). Predicting emergence and first flight of bark beetle species can facilitate timing of management strategies, such as monitoring, single tree protection treatments and silvicultural activities.

The ponderosa pine forests (*Pinus ponderosa* Douglas ex. Lawson) of northern Arizona are home to multiple species of bark beetles including; *Dendroctonus brevicomis* LeConte, *D. frontalis* Zimmermann, *D. adjunctus* Blandford, *D. approximatus* Dietz and *D. valens* LeConte , *Ips pini* (Say), *I. latidens* (LeConte), *I. lecontei* Swaine, *I. knausi* Swaine and *I. calligraphus* (Germar) (Wood 1982, Villa-Castillo and Wagner 1996, Sanchez-Martinez and Wagner 2002, McHugh et al. 2003, Gaylord et al. 2006). *D. ponderosae* Hopkins has also been reported in trapping studies, however numbers appear to be limited and ponderosa pine tree mortality from this species in Arizona south of the Grand Canyon is negligible (Sanchez-Martinez and Wagner 2002, McHugh et al. 2003, Annual Insect and Disease Aerial Detection Survey maps of the USDA Forest Service: Region-3, Flagstaff Forest Health Protection, 1976-2000. USDA, Forest Service, Region 3, Forest Health Protection, Insect and Disease Condition Reports 2001-2003).

Minimum flight thresholds and optimum temperatures for flight have been reported in other geographic locations for some of the beetle species present in northern Arizona. In the northwestern US, ambient air temperatures for initial flights of *I. pini* are reported as being between 15.6-21.1 °C (Livingston 1979). For *D. brevicomis* in California this threshold has been reported as 15.6 °C (Miller and Keen 1960). Under field conditions in Louisiana flight thresholds as low as 6.7 °C have been reported for *D. frontalis* (Thompson and Moser 1986). However, there is a paucity of information on flight temperatures required for these species and others in the Southwest. Few studies report degree day requirements for beetle development (Ayers et al. 2001, Kennedy and McCullough 2002) and none of these are geographically specific to northern Arizona. Similarly, to the best of our knowledge, there is no data available on temperature requirements for flight or development for *Enoclerus sphegus* Fabricus (Coleoptera: Cleridae), *E. lecontei* (Wolcott) and *Temnochil.chlorodia* (Mannerheim) (Coleoptera: Ostomidae), the three most prominent bark beetle predators in northern Arizona.

Our goal is to determine predictive tools of bark beetle flight for species occurring in ponderosa pine forests of northern Arizona. We examine both degree day data and temperature thresholds reached prior to bark beetle flights. In addition, we examine if beetle flight is most highly correlated with maximum, minimum or average temperatures. This information will provide managers with an easily accessible tool to predict flight initiation and periods of beetle flight activity. In addition, this information may help predict what impact increasing global temperatures could have on bark beetle populations including their interactions with predators and interspecies competition (Ayers et al. 2001, Williams and Liebhold 2002, Aukema et al. 2005, Logan et al 2006).

MATERIALS AND METHODS

Site Description. Our study sites were located across the ponderosa pine forest type in north central Arizona. Our study used trap catch data from seven different sites at three different elevations; low (1,676 m ± 76 m), mid (2133 m ± 76 m), and high (2,591m ± 76 m). At upper elevations, forest composition includes ponderosa pine, Douglas fir (*Pseudotsuga menziesii* Mirbel), limber pine (*Pinus flexilis* James), and quaking aspen (*Populus tremuloides* Michx.). At middle elevations, forest overstory is comprised of ponderosa pine, which occurs in near monocultures and Gambel oak (*Quercus gambelii* Nutt.). At lower elevations, ponderosa pine stands are smaller and interspersed with alligator bark juniper (*Juniperus deppeana* Steud.), Arizona white oak (*Quercus arizonica* Sarg.) and open grassland.

Study Design. To assess bark beetle flight activity with regard to air temperatures we... In 2002 and 2003 our study was conducted at one mid-elevation site. where we used 2 clusters of 5, 8-unit Lindgren funnel traps (Phero Tech Inc., Delta, BC, Canada) (Lindgren 1983) in a pentagon-shaped arrangement with a different lure type for each trap in a cluster (Table 1). Traps were monitored from January 1, 2002 through December 31st, 2003. In 2005 and 2006 our study was conducted at five low-elevation, six mid-elevation and six high- elevation sites. Each site consisted of a triangular arrangement of three twelve-unit Lindgren funnel traps (Synergy Semiochemicals Corp., Burnaby, BC, Canada) (Lindgren 1983), baited with a different lure type (Table 1). Traps in 2005 and 2006 were monitored from March or April (depending on elevation and snowpack) through November. Lures at all sites were changed regularly using Phero Tech guidelines. Although we used a different sterioisomeric ratio of ipsdienol in the *I. pini* lure in 2002 and 2003 than in 2005 and 2006, we feel this should not influence early season trap catches as previous research (Steed 2003) has shown that isomeric specificity was not present in *I. pini* in northern Arizona until the second flight (June/July). Traps were hung on 3 m

conduit poles with the bottom of traps ~1 m from the ground and placed a minimum of 15m apart. Traps were rotated on a regular basis to minimize location impacts. A small piece of insecticide (18.6% Dichlorvos, United Industries Corp., St. Louis, MO, USA) was placed in each collection cup to kill trapped insects and minimize predation.

Beetles and their associated predators were collected from traps every one to two weeks and were sorted to species according to Furniss and Carolin (1977), Chansler (1964) and Wood (1982). Species identifications were confirmed using voucher specimens in the lab, genetic sequencing conducted at the Center for Microbial Genetics and Genomics at Northern Arizona University and from samples shipped to Drs. John Moser, USDA Forest Service Southern Research Station and Anthony Cognato, Texas Agricultural and Mechanical University. Voucher specimens are maintained in the entomology lab at Northern Arizona University and the Rocky Mountain Research Station, United States Forest Service, Flagstaff, Arizona.

Temperature data were recorded using a Campbell Data logger and shaded thermocouple, located approximately 1.4 m above the-ground, which measured air temperature every 30 minutes (2002 and 2003) and HOBO data loggers (Onset Computer Corp., Bourne, MA, USA) which were installed on the north side of a tree nearest to the center of the plot and recorded temperature once every one or three hours throughout the trapping season (2005 and 2006). Not all temperature data sets were complete due to occasional battery failure or malfunction of the data loggers.

Statistical analysis. Based on our temperature data, we used actual day-degree accumulation (Pruess 1983), i.e. the sum of the degrees by day exceeding the minimum developmental threshold (established as base 11°C based on studies from Miller and Keen 1963 and Ungerer et al. 1999 for development rates of bark beetles) from January 1st to first beetle

capture. For each species we analyzed degree days for those locations and years where we captured at least 60 beetles for the season and temperature loggers were installed prior to January of the respective year. To test for significant differences in degree day accumulation for each species by site and year we used a Chi-squared analysis. The null hypothesis was no difference in degree day accumulation between the sites, i.e. the expected value at each site was the average of the degree day sum of all sites. Significance was established at $\alpha = 0.05$.

To assess temperature thresholds we analyzed the average number of beetles captured by species by the maximum daily temperature for each trapping period. For those species for which we had at least four sites where more than 50 individuals of the respective species were captured over the season and, where traps were deployed for at least two week prior to first beetle capture (indicating we captured the first beetles flying for the season) we used paired t-test analysis to analyze minimum, average and maximum temperatures for the weeks prior to first flight and the week of first flight.

RESULTS

Degree days. For all species analyzed degree days were significantly different ($p < 0.0001$) among sites (Table 2).

Threshold temperature. *Ips pini*: We collected a total of 14,398 *I. pini*. We captured beetles when temperatures exceeded 15 °C, however, greatest trap catches only occurred when maximum daily temperatures exceeded 17 °C (Fig. 1a). The minimum (df =8 , t value = -2.11, p = 0.0680) average (df =8 , t value = 5.41, p = 0.0006), and maximum (df =8, t value = 10.20, p < .0001) temperatures of all sites was slightly higher for the first week of the year in which beetles were collected than the week prior (Fig. 2 or TABLE 3). The lowest maximum temperature at

any site for initial beetle flight was 16.1 °C. The lowest maximum temperature at which *I. pini* was captured was 11.7 °C in March of 2006 (Fig. 1a).

Ips lecontei: We collected a total of 1,942 *I. lecontei*. We captured beetles when maximum temperatures exceeded 15 °C, however, greatest trap catches only occurred when maximum daily temperatures exceeded 19 °C (Fig. 1b). The lowest maximum temperature for any trapping period during which *I. lecontei* was captured was 15.2 °C which occurred in March. Due to low beetle catches at some sites and the lack of temperature data prior to first seasonal beetle collection we were unable to analyze data for species in terms of temperature for first yearly flight.

Ips calligraphus: We collected a total of 2,680 *I. calligraphus*. At all sites and years few *I. calligraphus* were collected until maximum daily temperatures approached 20 °C (Fig. 1c). The lowest maximum temperature for any trapping period during which *I. calligraphus* was captured was 15.5 °C in October. Due to low beetle catches at some sites and the lack of temperature data prior to first seasonal beetle collection we were unable to analyze data for species in terms of temperature for first yearly flight.

Dendroctonus brevicomis: We collected a total of 6,859 *D. brevicomis*. The majority of *D. brevicomis* were captured when temperatures exceeded 15 °C (Fig. 3a). The minimum temperature for all sites did not differ between the week prior to first beetle capture and the week of first beetle capture (df = 12, t value = .94, p = 0.3639) (Fig. 4a or Table 3). The average (df =12, t value = 2.65, p = 0.0213) and maximum (df =12, t value = 4.21, p = 0.0012) temperatures of all sites was higher for the first week of the year in which beetles were collected than the week prior. The lowest maximum temperature at any site for initial beetle flight was 18.6 °C,

however, later in the season (October), *D. brevicomis* was captured when maximum temperatures for the trap period did not exceed 13.1 °C (Fig. 3a).

Dendroctonus frontalis: We collected a total of 13,091 *D. frontalis*. The majority of *D. frontalis* were captured when maximum temperatures exceeded 15 °C (Fig. 3b). The minimum temperature for all sites did not differ between the week prior to first beetle capture and the week of first beetle capture (df = 14, t value = 1.44, p = 0.1709) (Fig. 4b or Table 3). However, the average and maximum temperatures of all sites was higher for the first week of the year in which beetles were collected than the week prior (df =14, t value = 4.59, p = 0.0004 and df =14, t value = -5.30, p = 0.0001, respectively). The lowest maximum temperature at any site for initial beetle flight was 17.8 °C, however, later in the season (November), *D. frontalis* was captured when maximum temperatures for the trap period did not exceed 10.4 °C (Fig. 3b).

Dendroctonus valens: We collected a total of 1,898 *D. valens*. The majority of *D. valens* were captured when maximum ambient temperatures exceeded 17 °C (Fig. 3c). There was no difference between the minimum temperatures for the week prior to first beetle capture and the week of first beetle capture (df = 7, t value = 0.30, p = 0.7714) (Fig. 4c or Table 3). There was a significant difference between the average (df = 7, t value = -5.55, p = 0.0009) and maximum (df = 7, t value = -5.44, p = 0.0010) temperatures for the week prior to and the week of first beetle capture. The lowest maximum temperature at any site for initial beetle flight was 16.1 °C (March) which was also the lowest maximum temperature for a capture period for *D. valens* (Fig. 3c).

Dendroctonus adjunctus: We collected a total of 7,044 *D. adjunctus*. *D. adjunctus* was captured in traps when maximum temperatures exceeded 12 °C and the majority (81%) of beetles were collected when temperatures were below 30 °C (Fig. 3d). There was no difference between

the minimum temperatures for the week prior to and the week of first beetle collection average (df =7, t value = .85, p = 0.4259) (Fig. 4d or Table 3). There was a significant difference between the average (df = 7, t value = -2.78, p = 0.0274) and maximum (df = 7, t value = -4.66, p = 0.0023) temperatures for the week prior to and the week of first beetle capture. The lowest maximum temperature at any site for initial beetle flight was 14.5 °C; however, later in the season (November), *D. adjunctus* was captured when maximum temperatures for the trap period did not exceed 11.4 °C (Fig. 3d).

Dendroctonus approximatus: We collected a total 2,908 *D. approximatus*. The majority of *D. approximatus* were captured when temperatures exceeded 17 °C (Fig. 3e). There was no difference between the minimum temperature for the week prior to and the week of first beetle capture (df =11, t value = .82, p = 0.4281) (Fig. 4e or Table 3). Average temperatures and maximum temperatures were higher for the first beetle capture period compared to the trap collection prior to first beetle capture (df =11, t value = 3.95, p = 0.0023 and df =11, t value = 3.81, p = 0.0029 for average and maximum, respectively). The lowest maximum temperature at any site for initial beetle flight was 17.5 °C and 16.3 °C was the lowest maximum ambient temperature recorded for any trapping period when *D. approximatus* was captured (April) (Fig. 3e).

Enoclerus spp: We collected a total of 3,759 *Enoclerus* The majority of *Enoclerus* were captured when temperatures exceeded 16°C (Fig. 5a). Minimum, average temperatures and maximum temperatures were higher for the first beetle capture period compared to the trap collection prior to first beetle capture (df =12, t value = 2.52, p = 0.0269, df =12, t value = 6.53, p < 0.0001 and df =12, t value = 7.21, p < 0.0001 for minimum, average, and maximum, respectively) (Fig. 6a or Table 3). The lowest maximum temperature at any site for initial beetle

flight was 17.4 °C; however, in May of 2005 beetles were captured when maximum temperatures for a trap period did not exceed 10.2 °C (Fig. 5a).

Temnochila chlorodia: We collected a total of 7,518 *T. chlorodia*. The majority of *T. chlorodia* were captured when the maximum ambient temperature for a capture period exceeded 20°C (Fig. 5b). Minimum, average temperatures and maximum temperatures were higher for the first beetle capture period compared to the trap collection prior to first beetle capture ($df = 20$, t value = 2.36, $p = 0.0286$, $df = 20$, t value = 6.60, $p < 0.0001$ and $df = 20$, t value = -4.24, $p < 0.0001$, for minimum, average and maximum, respectively) (Fig. 6b or Table 3). The lowest maximum temperature at any site for initial beetle flight was 19 °C; however, in March of 2005 *T. chlorodia* was captured when maximum temperatures for a trap period did not exceed 16 °C (Fig. 5b).

DISCUSSION

Our data suggests that degree days for most species are not a reliable method for predicting the onset of beetle flight in the southwestern US. Multiple sites recording beetle flight and temperature, and a more thorough experimental design which includes date of last fall oviposition and temperature data from oviposition to first beetle flight is necessary to verify this conclusion. In addition, temperatures in the phloem are more reflective of the actual conditions experienced by developing larvae (Werner and Holsten 1985) and potentially over-wintering adults. While recent research suggests that the subcortical temperature of un-attacked ponderosa pine only differs from ambient temperature by 1-2 °C (Gaylord, unpublished data) previous studies suggest that the phloem temperature in attacked trees may differ more substantially from ambient air temperatures (Beal 1934, Schmid et al. 1993).

Our data generally corresponds with previous studies from other geographic regions for flight initiation of bark beetle species. *Ips pini* began flights in our study when temperatures exceeded 15 °C corresponding with the reported range of 15.6 – 21.1 °C reported for onset of flight activity in the Northwest (Livingston 1979). For *D. brevicomis*, the range of temperature for flight activity has been reported as 7.2-35 °C (Miller 1931, Miller and Keen 1960), our study showed spring beetle activity beginning when temperatures exceeded 18.6 °C and continuing in the fall until temperatures dipped below 13 °C. For *D. frontalis* flight usually occurs between 22-36.7 °C (White and Franklin 1976, Greer et al. 1981, Thompson and Moser 1986) but has been found as low as 6.7 °C (Thompson and Moser 1986). Our study found spring flight initiating at 16.1°C, but later season flights continued when temperatures were as low as 10.4 °C (Fig. 4b). The lowest minimum flight for any scolytid is 6.7 °C by *D. frontalis* in Louisiana (Thompson and Moser 1986). Emergence for *D. adjunctus* has been reported as low as 4.4 °C (Lucht et al. 1974), however, temperature requirements for emergence may be lower than those for flight initiation. Our study found *D. adjunctus* flying in the spring when temperatures reached 14.5 °C but later in the fall, flights continued when maximum daily temperatures did not exceed 11.4 °C.

Our data also indicate that flight activity for *I. pini* appears to be strongly correlated with a temperature threshold and does not have strong degree day requirements or reliance on some other environmental cue for beetles to become active. For instance, in 2003 *I. pini* was captured in all months other than February and December (Gaylord et al. 2006). In addition, during the first week of trap collection for 2006 (March 2nd – March 7th) maximum recorded temperatures at the low-elevation sites ranged from 19.0 – 20.2 °C and *I. pini* beetles were captured at all sites. Temperatures then declined (maximum recorded temperatures ranged from 14.1 to 16.0 °C) for

the next two weeks (March 8th- March 21st) and no beetles were captured. However, when temperatures once again warmed, 19.0 – 22.1 °C for March 22nd through March 28th, captures resumed.

In contrast to *I. pini*, only three *D. brevicomis* were captured across all sites during the first week of March. No other *Dendroctonus* were collected until the March 28th collection, when temperatures warmed again. Therefore, we suggest that the majority of the *Dendroctonus* beetles, while sensitive to temperature, also require some other cue before flight occurs in the spring. Some of the differences we observed in terms of flight initiation in response to increases in temperature could be due to differences in overwintering biology of different beetle species and/or ability to supercool (Lombardero et al. 2000). While research from other regions has shown that *D. frontalis* and *D. brevicomis* (Wood 1982) may overwinter in all stages, including the adult, and therefore might be able to initiate flight as soon as temperatures warm, this has not been verified in our region and anecdotal reports (McMillan personal observation) indicate that most *D. brevicomis* in northern Arizona over winter in the larval stage. Tran et al. (2007) observed that the dominant over wintering stage of *D. frontalis* in New Jersey, the most northern range of the *D. frontalis*, is the final instar larvae that have completed feeding.

For the two genera of predators we monitored, *Enoclerus spp.* and *T. chlorodia*, threshold temperatures for flight appeared to be similar to bark beetle species, ranging between 15-20 °C. However, *T. chlorodia* seemed have a warmer threshold (approximately 20 °C) for flight than most of the bark beetle species and *Enoclerus spp.*, which corresponds to previous reports that peak *T. chlorodia* flight tends to be later in the season than most bark beetle species (Gaylord et al. 2006). Because we did not separate to the species level, results for *Enoclerus* should be interpreted with caution.

Our data indicate that for many beetle species, once the crucial flight temperature threshold has been crossed in a season, beetles may subsequently remain active at lower temperatures. For instance, the lowest maximum temperature at any site for initial flight of *I. pini* was 16.1 °C; however, at six different sites beetles were captured when temperatures were lower than this threshold (ranging from 11.7°C to 15.9 °C). This observation also held true for all the *Dendroctonus* species, with the exception of *D. valens*, and the two predator genera as later in the season we captured beetles when maximum temperatures for the capture period were lower than our hypothesized lowest initial flight threshold.

The relationship between *D. adjunctus* trap captures and temperature was unique among the beetle species with more flights occurring when temperatures were cooler. In fact, this is the only species where an argument could be made for an upper temperature threshold as our data indicates that most (81%) beetles were captured when maximum temperatures are below 30 °C (Fig. 3d). *D. adjunctus* is a univoltine species and shows strong flight synchrony with the majority of beetles captured in funnel traps over a two or three week period in the fall (Chansler 1967, Massey et al. 1977, Wood 1982, Gaylord et al. 2006). Further research is necessary to determine if these beetles respond to declining temperatures in the fall or if there are other environmental cues which trigger emergence and host searching.

For all species analyzed, other than *D. adjunctus*, it does not appear that the upper temperature for flight was exceeded. However, because our trap catch data were collected over a seven day period, it is impossible to verify on what day beetles were flying. Therefore, an unknown upper temperature threshold may have been exceeded on certain days. Similarly, beetles may have been flying at lower temperatures than our figures suggest as we presented the maximum recorded daily temperature for the capture period. Although no beetles were ever

collected when maximum temperatures for the collection period were below 10°C it is important to note that we actually only had four collection periods in all four years where a maximum temperature of at least 10°C was not reached at some point during the collection period.

Implications. Significant difference in average and maximum temperature between the week prior to beetle capture and the week of first beetle capture occurred for all bark beetle species and predators examined, whereas comparisons of differences in minimum temperatures were only significant for the predator species. Therefore, we conclude that initiation of beetle and predator flight can be predicted by monitoring maximum daily temperatures. Since daily maximum temperature is generally easy to obtain, this should be a simple to use tool for land managers.

Our research indicates that most beetle species are responsive to temperatures for flight initiation and, presumably, host seeking and attack. Other research clearly indicates that temperature changes could influence rates of development, number of generations per year, emergency synchrony, and distribution of bark beetles (Miller and Keen 1960, Wagner et al. 1984, Bentz et al. 1991, Ungerer et al. 1999, Logan and Bentz 2000, Hansen et al. 2001a, Williams and Liebhold 2002, Friedenberg et al. 2007). Therefore, warming temperatures could lead to shifts in seasonality of beetle flights with more activity in the spring and fall as well as more generations per year and, potentially, more bark beetle-caused tree mortality. Changes in temperature regimes may alter emergence patterns of offspring cohorts that influence beetle aggregation patterns and host colonization success (Tran et al. 2007, Friedenberg et al. 2007). In addition, increasing temperatures could lead to shifts in the current geographic distribution of beetles and their hosts (Williams and Liebhold 2002). However, while some beetles may increase their geographic range, other species' range could decline. In short, increasing temperatures could

benefit some bark beetle species and hinder others and interactions with changes in predator populations and or phenology as well as host range shift and/or phenology could confound the direct impacts of temperature on bark beetle development rates and seasonal flight initiation and cessation (Williams and Liebhold 2002, Aukema et al. 2005, Lange et al. 2006, Logan et al. 2006).

Acknowledgements

Thanks to C. Hayes, B. Loomis, T.S. Davis, A. Garcia, V. Bunker, G. Hancock, K. Baumgartner, E. Osborne, W. Winslow, A. Miller, M. Moreno, L. Smith, K. Murray, M. Schaffer, and A. Somerville for assistance with field work. C. Allender, A. Cognato and J. Moser for assistance with beetle species identification. K. Wallin and T. Kolb for assistance with study design. Thanks also to K. Clancy for helpful review comments and E. Smith (Forest Health Technology Enterprise Team) for his support. Support for this research was provided by USDA Forest Service, Forest Health Technology Enterprise Team, National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (CREES) grant, Northern Arizona University, School of Forestry, Mission Research Program, the McIntire Stennis Program and the USFS FHP Western Bark Beetle Research Initiative and agreement 03-JV-11221605-237 between the RMRS and NAU, and National Fire Plan research project 01.RMS.B.4.

Reference List

Aukema, B. H, M. K. Clayton, and K. F. Raffa. 2005. Modeling flight activity and population dynamics of the pine engraver, *Ips pini*, in the Great Lakes region: effects of weather and predators over short time scales. Population Ecology 47: 61-69.

Ayres, B. D., M. P. Ayres, M. D. Abrahamson, and S. A. Teale. 2001. Resource partitioning and overlap in three sympatric species of *Ips* bark beetles (Coleoptera: Scolytidae). Oecologia 128: 443-453.

Beal, J. A. 1934. Relation of air and bark temperatures of infested ponderosa pines during subzero weather. Journal of Economic Entomology 27: 1132-1139.

Bentz, B. J., J.A Logan, and G. D. Amman. 1991. Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. Canadian Entomologist 123: 1083-1094.

Chansler, J. F. 1964. Overwintering habits of *Ips lecontei* Sw. and *Ips confusus* (Lec.) in Arizona and New Mexico. U.S. Forest Service Research Note RM-27: 1-4.

Chansler, J. F. 1967. Biology and life history of *Dendroctonus adjunctus* (Coleoptera: Scolytidae). Annals of the Entomological Society of America 60: 760-767.

Friedenberg, N.A., J.A. Powell, and M.P. Ayres. 2007. Synchrony's double edge: transient dynamics and the Allee effect in stage structured populations. Ecological Letters, *in press*.

Furniss, R. L. and V. M. Carolin. 1977. Western Forest Insects. U.S. Government Printing Office, Washington, D.C.

Gaylord, M. G., T. E. Kolb, K. F. Wallin, and M. R. Wagner. 2006. Seasonality and Lure Preference of Bark Beetles (Curculionidae: Scolytinae) and Associates in a Northern Arizona Ponderosa Pine Forest. Environ. Ent. 35(1): 37-47.

Greer S. F., J. E. Coster, and P. C. Johnson. 1981. Effects of weather on flight activity of southern pine beetle. Journal of the Georgia Entomological Society 16 (3): 272-282.

Hansen, E. M., B. J. Bentz, and D. L. Turner. 2001a. Physiological basis for flexible voltinism in the spruce beetle (Coleoptera: Scolytidae). The Canadian Entomologist 133: 805-817.

Hansen, E. M., B. J. Bentz, and D. L. Turner. 2001b. Temperature-based model for predicting univoltine brood proportions in spruce beetle (Coleoptera: Scolytidae). Canadian Entomologist 133: 827-841.

Higley, L. G., L. P. Pedigo, and K. R. Ostlie. 1986. DEGDAY: A program for calculating degree-days, and assumptions behind the degree-day approach. Environmental Entomology 15: 999-1016.

Holsten, E. H., and J. S. Hard. 2002. Dispersal flight and attack of the spruce beetle, *Dendroctonus rufipennis*, in south-central Alaska. United States Department of

Agriculture, Forest Service, Research paper PNW-RP-536.

Kennedy, A. A., and D. G. McCullough. 2002. Phenology of the larger European pine shoot beetle *Tomicus piniperda* (L.) (Coleoptera: Scolytidae) in relation to native bark beetles and natural enemies in pine stands. Environmental Entomology 31(2): 261-272.

Lange, H., B. Økland, and P. Krokene. 2006. Thresholds in the life cycle of the spruce bark beetle under climate change. Interjournal for Complex systems 1648.

Lindgren, B. S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). Canadian Entomologist 115: 299-302.

Livingston, L. R. 1979. The pine engraver, *Ips pini* (Say), in Idaho: life history, habits and management recommendations. Idaho Department of Lands, Forest Insect and Disease Control, Report. Rep. No 79-3.

Logan, J. A. and B. J. Bentz. 1999. Model analysis of mountain pine beetle (Coleoptera; Scolytidae) seasonality. Environmental Entomology 28: 924-934.

Logan, J. D., W. Wolesensky, and A. Joern. 2006. Temperature-dependent phenology and predation in arthropod systems. Ecological modeling.196: 471-482.

Lombardero, M. J., M. P. Ayres, B. D. Ayres, and J. D. Reeve. 2000. Cold tolerance of four species of bark beetle (Coleoptera: Scolytidae) in North America. Environmental Entomology 29: 421-432.

Lucht, D. D., R. H. Frye, and J. M. Schmid. 1974. Emergence and attack behavior of

Dendroctonus adjunctus Blandford near Cloudcroft, New Mexico. Annals of the Entomological Society of America. 67 (4): 610-612.

Massey, C. L., D. D. Lucht, and J. M. Schmid. 1977. Roundheaded Pine Beetle. Forest Insect & Disease Leaflet 155. USDA, Forest Service.

McCambridge, W. F. 1971. Temperature limits of flight of the mountain pine beetle, *Dendroctonus ponderosae*. Entomological Society of America Annals 64(2): 534-535.

McHugh, C. W., T. E. Kolb, and J. L. Wilson. 2003. Bark beetle attacks on Ponderosa Pine following fire in northern Arizona. Environmental Entomology 32: 510-522.

Miller, J. M. 1931. High and low lethal temperatures for the western pine beetle. Journal of Agricultural Research 43: 303-321

Miller, J. M. and F. P. Keen. 1960. Biology and Control of the Western Pine Beetle. . Washington, D.C., Pacific Southwest Forest and Range Experiment Station. Forest Service.

Netherer, S. and J. Pennerstorfer. 2001. Parameters relevant for modeling the potential development of *Ips typographus* (Coleoptera: Scolytidae). Integrated Pest Management Reviews 6: 177-184.

Powell, J. A., J. L. Jenkins, J. A. Logan, and B. J. Bentz. 2000. Seasonal temperature alone can synchronize life cycles. Bulletin of Mathematical Biology 62: 977-998.

Pruess, K. P. 1983. Day-degree methods for pest management. Environmental Entomology 12(3): 613- 619,

Sanchez-Martinez, G. and M. R. Wagner. 2002. Bark beetle community structure under four ponderosa pine forest stand conditions in northern Arizona. For. Ecol. Manage 170: 145-160.

Schmid, J. M., S. A. Mata, W. K. Olsen, and D. O. Vigil. 1993. Phloem Temperatures in Mountain Pine Beetle-infested Ponderosa Pine. USDA Forest Service RM-521: 10.

Steed, B. E. 2003. Factors affecting the ecology and management of *Ips pini* (Say) (Coleoptera: Scolytidae) in northern Arizona and western Montana. PhD. dissertation, Northern Arizona University, Flagstaff.

Thompson, W. A., and J.C. Moser. 1986. Temperature thresholds related to flight of *Dendroctonus frontalis* Zimm. (Col.: Scolytidae). Agronomie 6: 905-910

Tran , J.K., T. Ylioja, R.F. Billings, J. Regniere, and M.P. Ayres. 2007. Impact of minimum winter temperatures on the population dynamics of *Dendroctonus frontalis*. Ecological Applications 17(3): 882-899.

Ungerer, M.J., M.P. Ayres and M.J. Lombardero. 1999. Climate and the northern distribution limits of *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). Journal of Biogeography 26: 1133-1145.

USDA Forest Service, 2001-2003. Forest Insect and disease conditions in the United States. *Research Health Protection,* USDA Forest Service www.fs.fed.us/r3/resources/health/fid_surveys.shtml

Villa-Castillo, J. and M. R. Wagner. 1996. Effect of overstory density of *Ips pini* (Coleoptera: Scolytidae) performance in Ponderosa Pine Slash. Journal of Economic Entomology 89: 1537-1545.

Werner, R. A. and E. H. Holsten. 1985. Factors influencing generation times of spruce beetles in Alaska. Canadian Journal of Forest Research 15: 438-443.

Wagner, T.L., J.A. Gagne, P.J.H. Sharpe and R.N. Coulson. 1984. A biophysical model of southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), development. Ecological modeling 21: 125-147.

Williams, D.W. and A.M. Leibhold. 2002. Climate change and the outbreak ranges of two North American bark beetles. Agricultural and Forest Entomology 4: 87-99.

White, R. A., and R. T. Franklin. 1976. Activity of the southern pine beetle in response to temperature. Journal of the Georgia Entomological Society 11: 370-372.

Wood, S. L. 1982b. The Bark and Ambrosia Beetles of North and Central America (Coleoptera: Scolytidae), a Taxonomic Monograph: Great Basin Naturalist Memoirs, No. 6 Brigham Young University.

Zavlaski, V. A. 1988. Insect Development: Photoperiodic and temperature control.

Springer-Verlag.

TABLE 1: Lures and lure components used in 2002, 2003, 2005, and 2006 in ponderosa pine forests in northern Arizona. All lures were purchased from Phero Tech (Delta, BC, Canada).

2002 – 2003		2005-2006	
Lure Type	Components	Lure Type	Components
D. brevicomis	*exo*-brevicomin, frontalin, myrcene	*D. brevicomis*	(frontalin, exo-brevicomin, myrcene)
D. ponderosae	(myrcene, *exo*-brevicomin, *trans*-verbenol)	*I. pini*	(lanierone, ipsdienol +03/-97),
D. frontalis	(frontalin)	*I. lecontei*	(cis-verbenol +17/-83, ipsdienol +50/-50, ipsenol +50/-50)
D. valens	(*a*-pinene, *beta* pinene and 3-carene)		
I. pini	(lanierone, 50/50 ipsdienol)		

TABLE 2: Degree day accumulation (base 11) for all sites and years in a ponderosa pine forest in north central Arizona where we collected at least 60 bark beetle or bark beetle predator species for the season and temperature data were collected from January 1st of the respective year through the first beetle flight. Chi squared analysis was performed with H_0 = all sites equal to the average for the sum of all degree day data.

	Site (elevation) Centennial Forest 2002	Centennial Forest 2003	Freidlein Prairie 2006	Mormon Mtn South 2006	Saddle Mtn. 2006	Schultz Pass 2006	Marshall Lake 2006	Cinder Hills	P value
Species	**Degree Days from January 1st to first trap capture (Base 11)**								
Bark Beetles									
Dendroctonus brevicomis	183	133	-	-	-	841	*	230	< 0.0001
D. frontalis	183	114	-	-	-	380	*	346	< 0.0001
D. valens	221	66	128	-	-	380	*	*	< 0.0001
D. adjunctus	44	66	-	-	-	*	78	230	< 0.0001
D. approximatus	-	-	-	54	75	303	*	230	< 0.0001
I. pini	183	33	-	-	-	-	*	*	< 0.0001
I. lecontei	-	98	-	-	-	-	-	-	-
I. calligraphus	-	-	-	-	-	380	-	-	-
Predators									
Enoclerus	183	98	-	54	45	-	-	-	< 0.0001
Temnochila	595	240	-	136	-	464	-	346	< 0.0001

Table 3: Temperature mean ± SD and (range) for the minimum, average and maximum temperatures for the week prior to flight and the week of first seasonal flight for bark beetle species and bark beetle predators in 2002, 2003, 2005 and 2006 in northern Arizona ponderosa pine forests.

	I. pini Mean (Range)	*D. brevicomis* Mean (Range)	*D. frontalis* Mean Range	*D. valens* Mean Range	*D adjunctus* Mean Range	*D. approximatus* Mean Range	*Enoclerus spp.* Mean Range	*T. chlorodia* Mean Range
Preflight Minimum (°C)	-6.5 ± 1.0 (-13.5 to -3.4)	-3.4 ± 1.5 (-13.5 to 6.2)	-5.1 ± 0.8 (-13.5 to -0.2)	-3.6 ± 1.0 (-9.0 to 0.2)	-5.9 ± 0.9 (-10.4 to -3.4)	-4.7 ± 0.6 (-7.5 to -1.1)	-3.9 ± 1.3 (-13.5 to 7.0)	- 3.2 ± 0.5 (-7.3 to 1.1)
1st flight Minimum (°C)	-4.1 ± 5.6 (-7.5 to -2.0)	-2.1 ± 1.1 (-10.4 to 5.0)	-3.8 ± 0.6 (-10.4 to -1.4)	-3.9 ± 1.5 (-14.0 to 0.3)	-7.0 ± 2.1 (-18.3 to -1.5)	-4.1 ± 0.8 (-10.4 to -1.5)	-1.6 ± 1.2 (-7.5 to 7.8)	-2.1 ± 0.4 (-7.3 to 2.0)
Preflight Average (°C)	3.2 ± 0.5 (1.0 to 4.9)	8.1 ± 1.4 (1.8 to 17.9)	5.9 ± 0.6 (1.5 to 9.4)	6.8 ± 1.1 (0.2 to 10.2)	4.3 ± 1.3 (0.2 to 10.5)	5.5 ± 0.6 (2.0 to 8.9)	6.8 ± 1.2 (1.6 to 18.0)	7.4 ± 0.5 (1.4 to 11.0)
1st flight Average (°C)	6.1 ± 0.6 (2.4 to 8.4)	10.3 ± 1.1 (2.0 to 16.7)	8.4 ± 0.6 (2.0 to 12.6)	8.3 ± 1.1 (1.4 to 12.8)	6.0 ± 1.6 (0.3 to 14.1)	7.5 ± 0.6 (2.7 to 10.9)	9.6 ± 1.2 (4.4 to 19.6)	9.6 ± 0.6 (2.4 to 15.9)
Preflight Maximum (°C)	15.5 ± 0.7 (13.6 to 18.9)	21.4 ± 1.4 (14.5 to 32.3)	19.3 ± 0.7 (14.1 to 23.2)	18.7 ± 1.5 (11.0 to 22.9)	14.6 ± 1.4 (10.2 to 22.9)	18.0 ± 1.0 (12.6 to 24.0)	19.7 ± 1.4 (13.7 to 29.5)	20.5 ± 0.8 (14.1 to 29.1)
1st flight Maximum (°C)	19.0 ± 0.6 (16.1 to 21.3)	23.8 ± 1.1 (18.6 to 32.8)	22.1 ± 0.8 (17.8 to 27.9)	21.8 ± 1.3 (16.1 to 26.7)	17.8 ± 1.1 (14.5 to 24.0)	20.7 ± 0.8 (17.5 to 24.8)	22.8 ± 1.5 (17.4 to 34.4)	23.3 ± 0.7 (19.0 to 30.7)

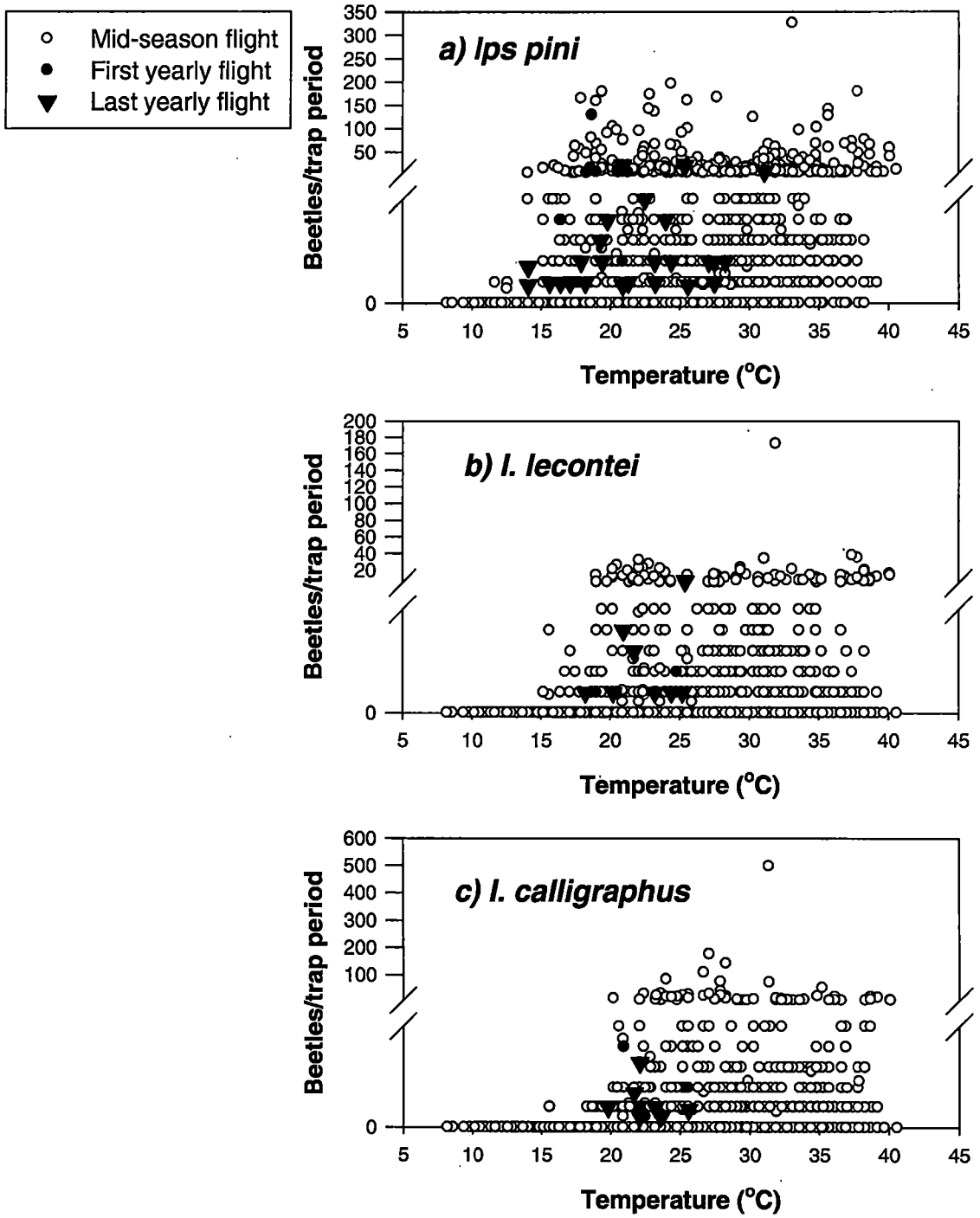


Figure 1: Scatter plot showing maximum temperature for first, mid and last seasonal trap periods for a) *Ips pini*, b) *I. lecontei*, and c) *I. calligraphus* beetles captured in 2002, 2003, 2005 and 2006 in ponderosa pine forests in northern Arizona. Y-axis scale varies among graphs.

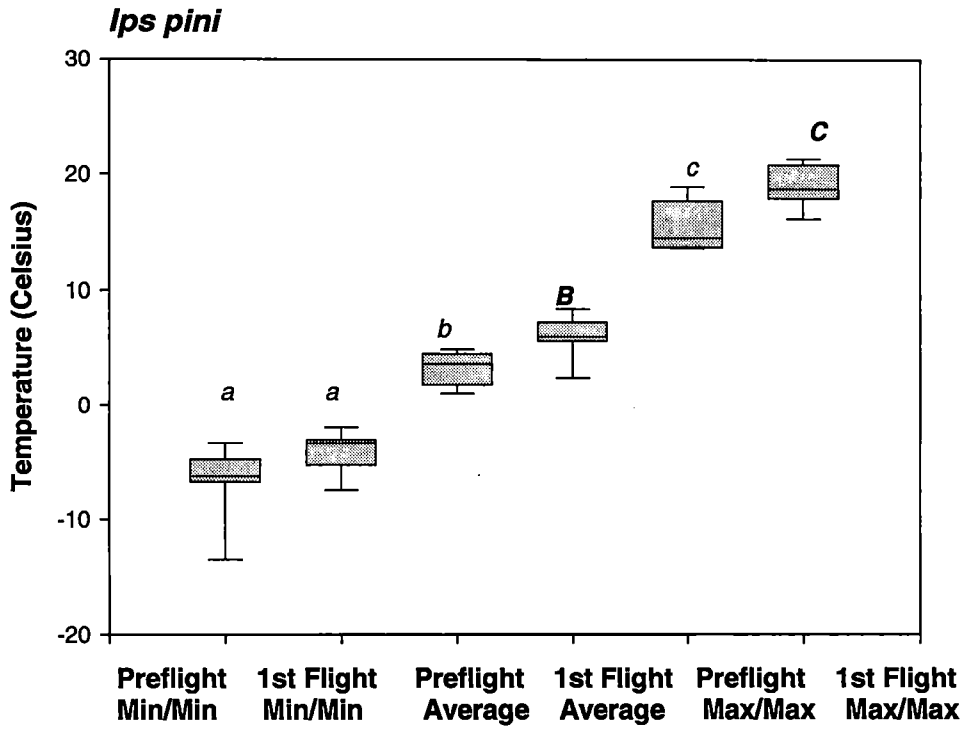


Fig. 2: Temperature range for the minimum, average and maximum recorded temperatures at nine sites in a ponderosa pine forest in north central Arizona for the capture period prior to first Ips pini capture (preflight) and during first beetle capture (1st flight) for the year. The center line in each box represents the median, upper and lower box boundary represents the 75th and 25th percentile respectively and error bars indicate 90th and 10th percentile. Paired t-tests were performed on columns with similar letters. Significant differences (α= .05) are indicated by lower case and capital letters.

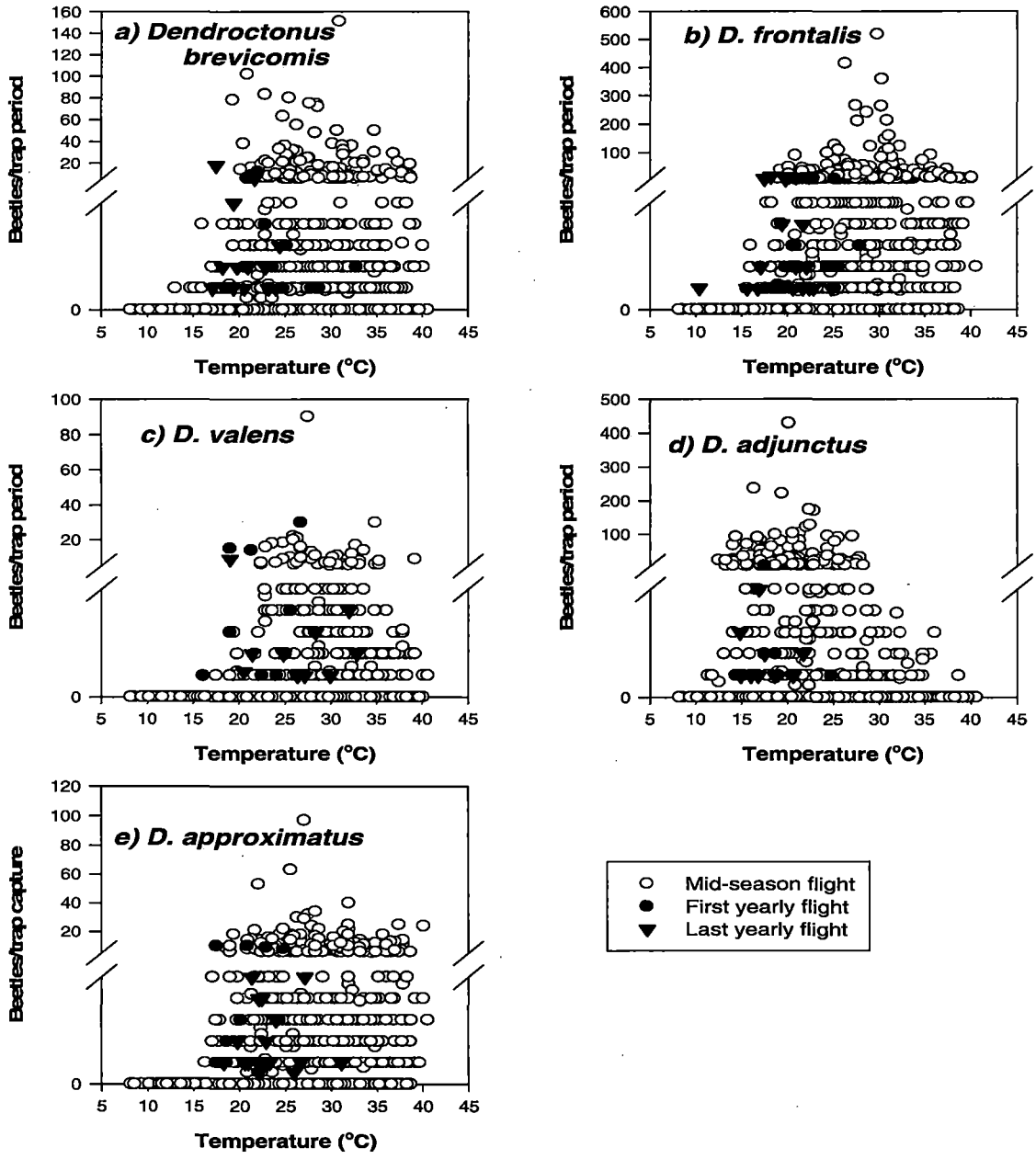


Figure 3: Scatter plot showing maximum temperature for first, mid and last seasonal trap periods for a) *Dendroctonus brevicomis*, b) *D. frontalis*, c) *D. valens,* d) *D. adjunctus,* and e) *D. approximatus* beetles captured in 2002, 2003,2005 and 2006 in ponderosa pine forests in northern Arizona. Y-axis scale varies among graphs.

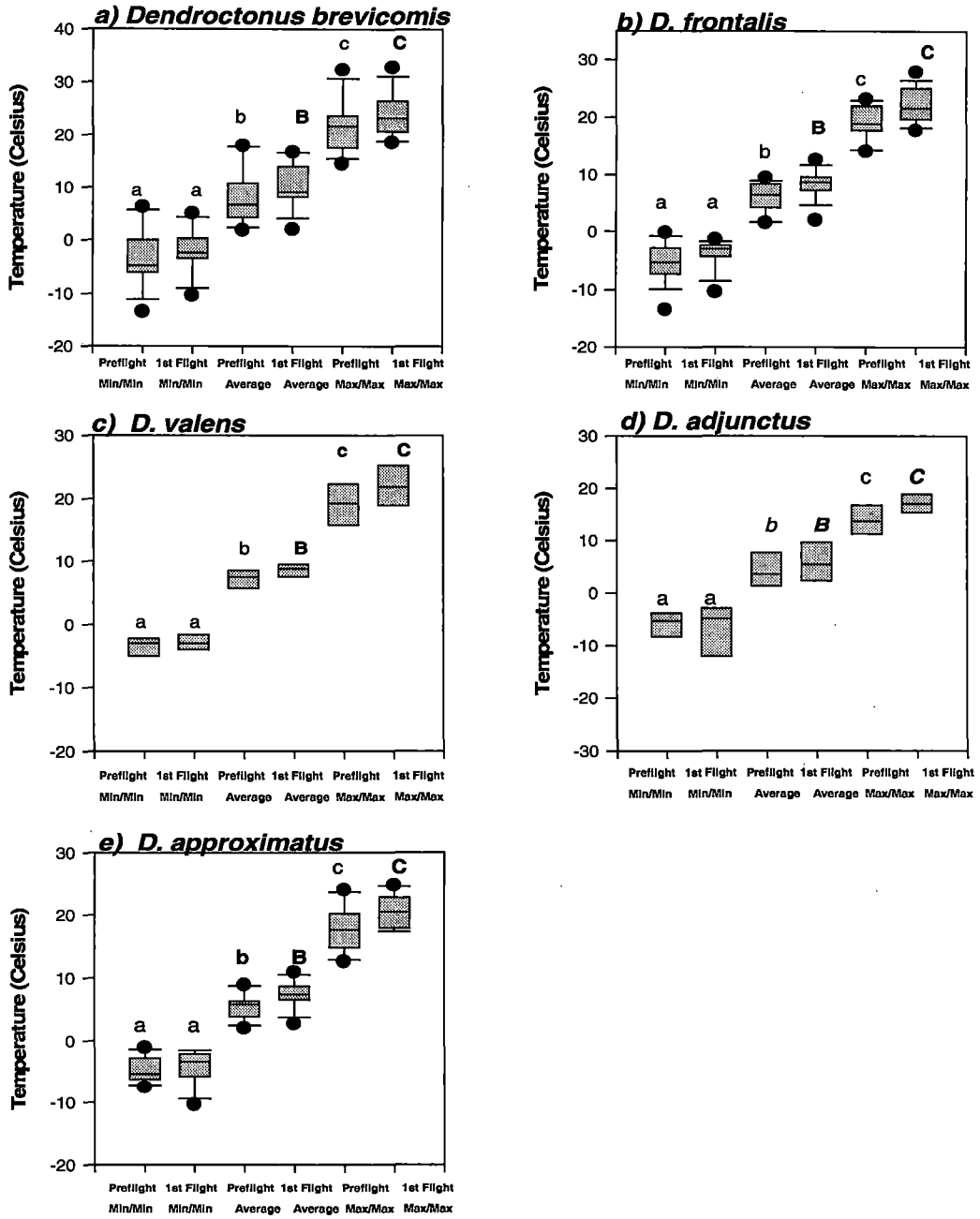


Fig. 4 Temperature range for the minimum, average and maximum recorded temperatures in a ponderosa pine forest in north central Arizona at a) 13, b) 15, c) eight, d) eight, and e) 12, sites for the capture period prior to first beetle capture (preflight) and during first beetle capture (1st flight). Paired t-tests were performed on columns with similar letters. Significant differences ($\alpha = 0.05$) are indicated by lower case and capital letters. The center line in each box represents the median, upper and lower box boundary represents the 75th and 25th percentile respectively and error bars indicate 90th and 10th percentile. Dots represent outliers.

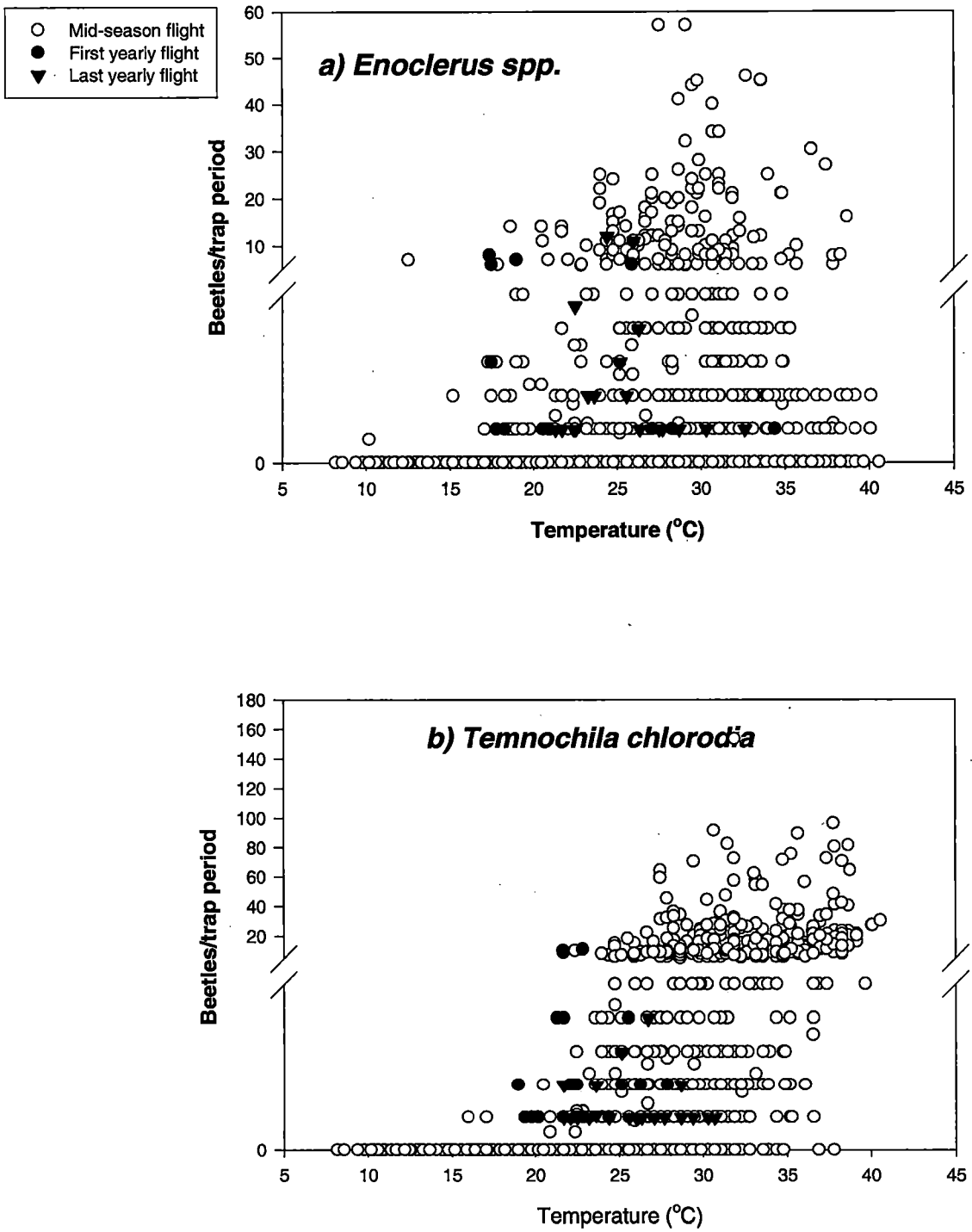


Figure 5: Scatter plot showing maximum temperature for first, mid and last seasonal trap periods for a) *Enoclerus* sp. and b) *T. chlorodia* beetles captured in 2002, 2003, 2005 and 2006 in ponderosa pine forests in northern Arizona. Y-axis scale varies among graphs.

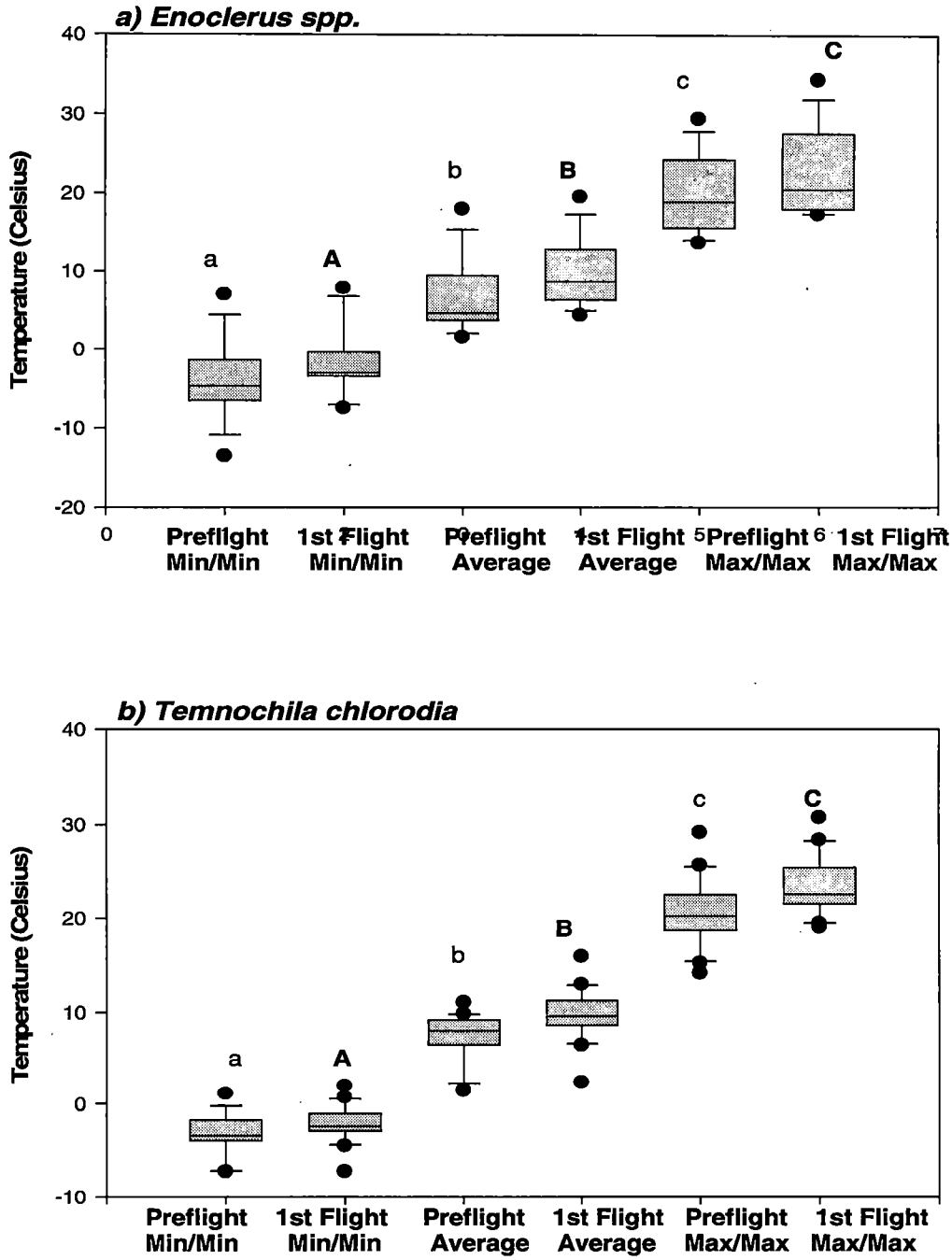


Fig. 6: Temperature range for the minimum, average and maximum recorded temperatures at a) 13, and b) 21, sites in a ponderosa pine forest in north central Arizona for the capture period prior to first beetle capture (preflight) and during first beetle capture (1st flight). Paired t-tests were performed on columns with similar letters. Significant differences ($\alpha = .05$) are indicated by lower case and capital letters The center line in each box represents the median, upper and lower box boundary represents the 75th and 25th percentile respectively and error bars indicate 90th and 10th percentile. Dots represent outliers.

Prepared for –an ecological-forestry journal

April 2007

Attraction of the southern pine beetle, *Dendroctonus frontalis* to pheromones of the western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Curculionidae: Scolytinae) in an allopatric zone

Deepa S. Pureswaran[1], Richard W. Hofstetter[2], and Brian T. Sullivan[3]*

[1]Natural Resources Canada, Atlantic Forestry Centre, P.O. Box 4000, Regent Street, Fredericton, NB E3B 5P7, Canada

[2]School of Forestry, Box 15018, Northern Arizona University, Flagstaff, AZ 86011, USA

[3]USDA Forest Service, Southern Research Station, Pineville LA 71360, USA

***Corresponding author:**

Office: 928-523-6452

FAX: 928-523-1080

e-mail: rich.hofstetter@NAU.EDU

Introduction

Pheromone mediated communication in bark beetles (Coleoptera: Curculionidae: Scolytinae) enables host and mate location, aggregation, and resource partitioning (Wood 1982a). Interspecific interactions occur when heterospecific beetles (Svihra et al. 1980), predators (Reeve 1997) and parasitoids cue into a colonized resource (Ayres et al. 2001; Dahlsten et al. 2004) that is usually rare or patchy in distribution. When two or more species are sympatric and inhabit the same tree, pheromones serve to partition the resource and minimize the deleterious effects of interspecific competition (Light et al. 1983; Rankin and Borden 1991) by maintaining adequate spacing among galleries. The genus *Dendroctonus* includes major killers of pine trees that often occur in outbreaks, during which they can overcome and kill healthy trees (Wood 1982b). Females initiate attack, excavate galleries in the phloem, and release aggregation pheromones that are attractive to both sexes (Borden et al. 1986; Raffa et al. 1993). They are joined by males that may also produce aggregation pheromones which further facilitate aggregation. Beetles mate and females lay eggs on the gallery walls after which antiaggregation pheromones are produced by both sexes, usually in higher amounts by males, terminating aggregation and switching attack to neighboring trees (Borden et al. 1986; Raffa et al. 1993).

Members of *Dendroctonus* spp. have common pheromone components, although the sex that produces them and their function varies. For example, frontalin is the female produced aggregation pheromone in the southern pine beetle, *D. frontalis* (Kinzer et al. 1969; Renwick and Vite 1969), the Douglas-fir beetle, *D. pseudotsugae* (Pitman and Vite 1970), and the spruce beetle, *D. rufipennis* (Dyer 1973; Dyer 1975).

However, in the western pine beetle, *D. brevicomis* it is a male produced aggregation pheromone (Kinzer et al. 1969), and in the mountain pine beetle, which is sympatric with all the above species, it is a male-produced (Ryker and Libbey 1982) multi-functional pheromone, facilitating aggregation in low doses and functioning in antiaggregation in high doses (Borden et al. 1987).

In Arizona, several bark beetle species occur in sympatry (Wood S.L. 1982; Gaylord et al. 2006), and *D. frontalis* and *D. brevicomis* often coexist on the same tree (Breece et al., in review; Hofstetter, pers. observ.). In the southern United States however, *D. brevicomis* is not found east of western Texas (DeMarsjr and Roettgering 1982), whereas D. frontalis occurs from East Texas eastward to the Gulf and Atlantic coasts and has a geographically isolated population in central Arizona (Payne 2006). During host colonization, female *D. frontalis* produce the aggregation pheromones frontalin and *trans*-verbenol when they land on a tree and initiate galleries, attracting both males and females (Payne et al. 1978). Males join females and produce *endo*-brevicomin (Pitman et al. 1969), which augments aggregation and may facilitate switching of attack focus to adjacent trees (Vité et al. 1985; Sullivan et al. 2007). Both sexes produce antiaggregation pheromones verbenone (predominantly males) and myrtenol which may function in terminating aggregation (Payne et al. 1978) and reducing intraspecific competition. In *D. brevicomis*, aggregation is mediated by female-produced *exo*-brevicomin (Silverstein et al. 1969; Browne et al. 1979; Byers 1983) and male produced frontalin (Kinzer et al. 1969). *endo*-Brevicomin has been reported to be produced by female *D. brevicomis* and may play a role in aggregation (Libbey et al. 1974).

Aggregation is terminated by *trans*-verbenol and verbenone produced by females and males, respectively (Renwick 1967).

There are conflicting reports on the role of *exo*-brevicomin in the behavior of *D. frontalis*. Vité et al. (1964) observed that volatiles emanating from *D. brevicomis*-infested pine bolts were attractive to *D. frontalis* in the field. However Payne et al. (1977) found that mixtures of *endo*- and *exo*-brevicomin reduced the attraction of *D. frontalis* to frontalin baits. Both species have common pheromone components (frontalin, *endo*-brevicomin, *trans*-verbenol, and verbenone), so one would expect that pheromones occurring in one species but not the other (e.g., *exo*-brevicomin produced by *D. brevicomis* but not *D. frontalis*) might mediate species-specificity in pheromone communication by either being attractive only to the producing species and/or repellent to the non-producing species, and thereby maintain reproductive isolation between the two species when they occur in sympatry. Investigation of cross-attraction of *D. frontalis* to *exo*-brevicomin in Arizona where they co-occur, revealed a significant increase in trap catch when *exo*-brevicomin was added to the pheromone bait of *D. frontalis* (Hofstetter et al., in review). This suggests that *D. frontalis* might use *exo*-brevicomin as an attractive cue to locate infested trees and raises the question of whether such cross-attraction persists in Mississippi where the two species are currently geographically isolated from each other.

We predicted that *D. frontalis* would not respond to *exo*-brevicomin outside of its zone of sympatry with *D. brevicomis*, therefore we conducted a field experiment to determine the effect *exo*-brevicomin on the behavior of *D. frontalis* in Mississippi. Additionally, to determine whether *D. frontalis* pheromone production differed inside and

outside the sympatric zone, we used gas chromatography-mass spectrometry (GC-MS) to examine the pheromone profiles of *D. frontalis* from Mississippi and Arizona, and compare these to pheromone profiles of *D. brevicomis* from Arizona. We performed gas chromatographic-electroantennographic detection analyses (GC-EAD) of the *exo*-brevicomin baits on the antennae of *D. frontalis* from Mississippi to confirm that this species was perceiving the *exo*-brevicomin in these baits. We also examined the effects of *exo*-brevicomin on the attraction of baited traps to *Thanasimus dubius* (Coleoptera: Cleridae), a predator of bark beetles in Mississippi.

Materials and Methods

GC-MS analyses of volatiles from beetles

Both sexes of *D. brevicomis* (n = 13 females; 10 males) and *D. frontalis* (n = 8 females; 3 males) were collected in flight traps near Flagstaff, Arizona in August 2005. Beetles were placed individually in 1 ml centrifuge tubes and shipped on ice to the USDA Forest Service, Southern Research Station in Pineville, Louisiana. Beetles that appeared healthy and vigorous were used in aerations to collect emitted volatiles. Volatiles were collected from individual beetles by confining them in 100 µl conical glass vials whose tips were filled with approximately 0.3 mg of adsorbent Super Q® (80-100 mesh) (Sullivan 2005). Individual beetles were inserted abdomen-first into the vials and immobilized using PFA tubing so that the tip of the abdomen was 1-2 mm from the adsorbent. Vials were loosely closed with a PTFE-lined cap to allow adequate gas exchange for beetle respiration. Volatiles released from the beetles were passively collected on the adsorbent Super Q® for 24 h at room temperature. A stream of purified, humidified air was passed over the

vials during incubation to avoid desiccation of beetles. Once aerations were complete, beetles were removed, and 50 µl redistilled pentane spiked with 3.5 ng / µl heptyl acetate (internal standard) was added to the adsorbent and allowed to sit for 15 min at room temperature. The supernatant was pipetted out, transferred to a GC autosampler vial and stored at -80°C for further analysis.

Gas chromatography-mass spectrometry (GC-MS)

Samples were analyzed on an Agilent 6890-5973 coupled gas chromatograph-mass spectral detector (GC-MS) using an HP-INNOWax (Agilent Technologies; 60 m x 0.25 mm x 0.25 µm film) column. The temperature program was 40° C for 1 min, 16° C / min to 80° C, then 7° C per min to 230° C and held for 10 min. Carrier gas (helium) flow was a constant 1.0 ml / min, and the injector and detector ports were held at 200 and 240° C respectively. The amounts of five major pheromones of *D. frontalis* and *D. brevicomis:* frontalin, *exo*-brevicomin, *endo*-brevicomin, *trans*-verbenol, verbenone and myrtenol (Payne et al. 1978) were quantified against a standard curve of detector responses to known concentrations of synthetic pheromones by comparing the relative abundance of diagnostic ions in analytes to the internal standard (MSD ChemStation software; G1701DA Version D.00.00.38, Agilent Technologies© 1989-2001). Means and standard errors of pheromone quantity isolated per beetle were calculated to compare their profiles between the two species.

GC-electroantennographic detection (GC-EAD) analyses of D. frontalis

We collected volatiles released from the *exo*-brevicomin baits utilized in field trapping studies in Mississippi. Two baits were placed into a sealed glass enclosure (50 ml) whose

inlet received air from an activated-charcoal filter and outlet was connected to a PFA cartridge containing conditioned Porapak Q (0.1 g; 50-80 mesh). Air (15 ml/min) was drawn through the enclosure and cartridge for 6 hr at 23° C, and then the cartridge was extracted with 1.5 ml redistilled pentane. This extract was then analyzed by GC-EAD using antennal preparations of *D. frontalis* reared from infested loblolly pine bolts collected within the Homochitto National Forest in western Mississippi. Techniques for antennal preparation and details of the GC-EAD apparatus are given in Sullivan (2005) and Asaro et al. (2004), respectively. The extract (1 µl) was injected split (1/20) onto an identical column as that used for the GC-MS analyses but with temperature program 80° C for 0.1 min, then ramped 4° C/min to 140° C, then 7 min at 230° C to purge the column. This program produced a 1.3 min separation between the elution of (and thus preparation exposure to) *exo*- and *endo*-brevicomin. The heights of signal voltage deflections in each GC-EAD run were corrected for time-dependent loss of responsiveness in the antennae by measuring the change in electroantennogram (EAG) responses to a mixture of *D. frontalis* semiochemicals at the beginning and end of each run (Sullivan et al., accepted). Corrected response voltages of five male and five female antennae responding to *exo*- or *endo*- brevicomin in the bait aeration were compared with a paired t-test. The relative proportions of *exo*- and *endo*- brevicomin in the bait aeration were determined by FID integration areas.

Field trapping studies

Attractive lures were acquired from Phero Tech Inc. and released from devices at the following rates (Table 1). The experiment employed twelve-unit multiple funnel traps (Lindgren 1983), set up in a straight line along the sides of logging roads, > 1 mile from

the nearest outbreak, in ten randomized complete blocks. Traps were ≥ 50 m apart, and were > 5 m from any pine to minimize possible effects on experiments of host volatiles or spill-over attacks. Treatments were: 1) turpentine 2) turpentine + frontalin 3) turpentine + *exo*-brevicomin and 4) turpentine + frontalin + *exo*-brevicomin. Captured beetles were frozen until they were identified, sexed (Osgood and Clark 1963) and counted. All data were transformed by $\log_{10}(x+1)$ to meet the assumptions of normality and homoscedasticity, and analysed by ANOVA and the Ryan-Einot-Gabriel-Welsch multiple range test (Day and Quinn 1989; SAS (1991-2000)). In all cases, $\alpha = 0.05$.

Results

GC-MS analyses of volatiles from beetles

The qualitative volatile profiles of *D. frontalis* and *D. brevicomis* are similar in that both species emit frontalin, *endo*-brevicomin, *trans*-verbenol, verbenone and myrtenol as previously reported (Figure 1). The main qualitative difference between the profiles of *D. brevicomis* and *D. frontalis* is that no *exo*-brevicomin was detected in either sex of *D. frontalis* from Arizona or Mississippi.

(Note differences in amounts of male verbenone between MS and AZ beetles. (Could do stats on relative amounts—For Mississippi frontalis, I took approximate data for males (n = 34) from Sullivan et al. 2007 (submitted, Fig 3) and data for females (n = 14) from Pureswaran et al. 2006).

GC-EAD analyses on exo-*brevicomin bait*

Consistent EAD peaks were recorded from male or female antennae only at the retention times of *exo*- and *endo*-brevicomin (Figure 2). Despite the very large disproportion

(108:1) of *exo*-brevicomin relative to *endo*-brevicomin in volatiles collected from the *exo*-brevicomin baits, the EAD voltages generated by *endo*-brevicomin were more than 50% those generated by *exo*-brevicomin. However, mean EAD responses to *exo*-brevicomin in the baits were significantly greater than to the contaminating *endo*-brevicomin for both females ($P = 0.027$) and males ($P = 0.002$).

Field-trapping

More *D. frontalis* of both sexes were caught in traps baited with turpentine + frontalin + *exo*-brevicomin than the other three treatments (Figure 3A,B) (males: $F_{3,27} = 26.85$, $P < 0.0001$; females: $F_{3,27} = 16.18$, $P < 0.0001$). For females, there were no differences among turpentine alone, turpentine + frontalin, and turpentine + *exo*-brevicomin (Figure 3A), whereas more males were captured in traps baited with turpentine + frontalin than turpentine alone or turpentine + *exo*-brevicomin (Figure 3B). The clerid predator *T. dubius* did not discriminate between traps baited with turpentine + frontalin or turpentine + frontalin + *exo*-brevicomin, but both those treatments caught more beetles than either turpentine alone or turpentine + *exo*-brevicomin (Figure 4A) ($F_{3,27} = 76.49$, $P < 0.0001$). The number of *Hylastes* sp. captured in traps baited with turpentine + *exo*-brevicomin was significantly different from turpentine alone ($F_{3,27} = 3.29$, $P = 0.0355$), but there were no significant differences among any of the other treatments (Figure 4B).

Discussion

Our study demonstrates that *D. frontalis* can perceive and respond positively to *exo*-brevicomin, an aggregation pheromone of a sympatric congener (*D. brevicomis*), at a

location hundreds of kilometers outside the sympatric zone (Figures 2, 3). It has previously been demonstrated that *D. frontalis* responds positively to *exo*-brevicomin within its zone of sympatry with *D. brevicomis* in Arizona (Gaylord et al. 2006, Hofstetter et al., in press). While females were not significantly more attracted to frontalin + turpentine than to turpentine alone, there was a three-fold increase in attraction with the addition of *exo*-brevicomin (Figure 3A). Males however, were significantly more attracted to turpentine + frontalin than to turpentine alone, and this attraction doubled with the addition of *exo*-brevicomin to the combination (Figure 3B). Turpentine + *exo*-brevicomin without frontalin were not attractive to either sex, indicating that there is a synergistic attractive function of *exo*-brevicomin when it occurs in combination with frontalin.

Both behavioral and electrophysiological data indicate that *D. frontalis* are highly sensitive to their aggregation pheromone *endo*-brevicomin (Sullivan et al., accepted). Simultaneously, commercially-available sources of *exo*-brevicomin are commonly contaminated with 1-2 % *endo*-brevicomin (David Wakarchuk, personal communication), as were our trap baits. Hence, this contamination could confound interpretation of studies of *D. frontalis* olfactory sensitivities and behavioral responses that employed synthetic *exo*-brevicomin. However, our GC-EAD results demonstrate that olfactory sensillae of *D. frontalis* are sensitive to *exo*-brevicomin when chromatographically freed of contamination, and that, at the quantitative proportions released from our trap baits, *exo*-brevicomin was a stronger olfactory stimulant than *endo*-brevicomin or any other contaminant. This suggests that beetle responses were, in fact, influenced disproportionately by the *exo*-brevicomin in our baits. Behavioral assays with *exo*-

brevicomin of extremely high purity may be necessary to completely eliminate the possibly confounding influence of *endo*-brevicomin contamination.

These results contrast with the early experiments of Vite and Renwick (1971) in which they found that *exo*-brevicomin inhibited the attraction of *D. frontalis* to frontalin + a-pinene. However, electroantennograms performed by Payne (1975) revealed no differences in the antennal responses of *D. frontalis* to *exo*-brevicomin compared to frontalin that suggested that *exo*-brevicomin might be inhibitory. In subsequent field tests by Payne et al. (1978), *exo*-brevicomin showed no inhibition to traps baited with frontalin + turpentine, leaving the semiochemical function of *exo*-brevicomin unresolved. Our results raise new practical and evolutionary questions on the role of *exo*-brevicomin on the behavioral ecology of *D. frontalis*. It also appears that addition of *exo*-brevicomin to the current lure might increase the efficiency of field trapping programs in the southeast United States.

Several studies have examined interspecific interactions and competition between bark beetles that co-attack the same tree. In most cases, when two or more bark beetle species attack the same tree, beetle responses to aggregation pheromones of their own species are inhibited by those of another species (Ayres et al. 2001), and this enables them to partition the tree and minimize interspecific competition (Byers and D.L.Wood 1980; Svihra et al. 1980; Light et al. 1983; Rankin and Borden 1991; Amezaga and Rodriguez 1998; Poland and Borden 1998). In the absence of pheromones from their own species, beetles may be attracted to aggregation pheromones of another species functioning as host-finding kairomones (Bowers and Borden 1992; Ayres et al. 2001).

The question of attraction of *D. frontalis* to pheromones of *D. brevicomis* in regions where populations of the two species are not sympatric remains to be answered. It is possible that the current eastern population of *D. frontalis* stems from an original western or southern (Mexico?) population where the two species co-occur. How pre-mating reproductive isolation mechanisms might separate the two species and enable them to find their mates when they attack the same tree in sympatric zones is another question that needs to be addressed (seminal rod structures, acoustic).

Acknowledgements

We thank Elisabeth Alden, Gabe DeGomez, Kevin Johnson, Laine Smith, and James Stewart for assistance in collecting and counting insects. We also thank Fool Hollow State Park, Apache/Sitgreaves NF, Coronado NF, Coconino NF, Prescott NF, and Tonto NF for trap collections. We thank two anonymous reviewers. This project was funded by Western Bark Beetle Initiative U.S.D.A. 05-PA-11221615-161, U.S.D.A. Cooperative Agreement 06-CA-11330129-046, and supported by School of Forestry Northern Arizona University and U.S.D.A. Forest Service.

References

Amezaga, I., and M. A. Rodriguez. 1998. Resource partitioning of four sympatric bark beetles depending on swarming dates and tree species. Forest Ecology and Management 109:127-135.

Asaro, C., B. T. Sullivan, M. J. Dalusky, and C. W. Berisford. 2004. Volatiles associated with preferred and nonpreferred hosts of the Nantucket pine tip moth, *Rhyacionia frustrana*. J. Chem. Ecol. 30: 977-990.

Ayres, B. D., M. P. Ayres, M. D. Abrahmson, and S. A. Teale. 2001. Resource partitioning and overlap in three sympatric species of *Ips* bark beetles. Oecologia 128:443-453.

Borden, J. H., D. W. A. Hunt, D. R. Miller, and K. N. Slessor. 1986. Orientation in Forest Coleoptera: An uncertain outcome of responses by individual beetles to variable stimuli. Pp. 97-110 *in* T. L. Payne, M. C. Birch and C. E. J. Kennedy, eds. Mechanisms in Insect Olfaction. Oxford University Press, New York.

Borden, J. H., L. C. Ryker, L. J. Chong, J. H.D. Pierce, B. D. Johnson, and A. C. Oehlschlager. 1987. Response of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. Can. J. For. Res. 17:118-128.

Breece, C.R., T.E. Kolb, B.G. Dickson, J.D. McMillin and K.M. Clancy. 2007. Prescribed fire effects on bark beetle activity and tree mortality in southwestern ponderosa pine forests. Forest Ecol. Manag. In review.

Browne, L. E., D. L. Wood, W. D. Bedard, R. M. Silverstein, and J. R. West. 1979. Quantitative estimates of the Western pine beetle attractive pheromone components, *exo*-brevicomin, frontalin, and myrcene in nature. J. Chem. Ecol. 5:397-414.

Byers, J. A. 1983. Influence of sex, maturity and host substances on pheromones in the guts of the bark beetles, *Ips confusus* and *Dendroctonus brevicomis.* J. Insect Physiol. 29:5-13.

Byers, J. A., and D.L.Wood. 1980. Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* LeConte and *Ips paraconfusus* Lanier, to their pheromones in the field. J. Chem. Ecol. 6:149-164.

Dahlsten, D. L., D. L. Six, D. L. Rowney, A. B. Lawson, N. Erbilgin, and K. F. Raffa. 2004. Attraction of *Ips pini* (Coleoptera: Scolyitdae) and its predators to natural attractants and synthetic semiochemicals in northern California: Implications for population monitoring. Environ. Entomol. 33:1554-1561.

Day, R. W., and G. P. Quinn. 1989. Comparison of treatments after an analysis of variance in ecology. Ecological Monographs 59:433-463.

DeMarsjr, C. J., and B. H. Roettgering. 1982. Western Pine Beetle. Forest Insect and Disease Leaflet 1. US Department of Agriculture Forest Service.

Dyer, E. D. A. 1973. Spruce beetle aggregated by the synthetic pheromone frontalin. Can. J. For. Res. 3:486-494.

Dyer, E. D. A. 1975. Frontalin attractant in stands infested by the spruce beetle, *Dendroconus rufipennis* (Coleoptera: Scolytidae). Can. Entomol. 107:979-988.

Gaylord, M.G., T.E. Kolb, K.F. Wallin, and M.R. Wagner. 2006. Seasonality and lure preference of bark beetles (Curculionidae: Scolytinae) and associates in a northern Arizona ponderosa pine forest. Environ. Ent. 35(1): 37-47.

Hofstetter, R.W., Z. Chen, M. Gaylord, J.D. McMillin and M.R. Wagner. 2007. Synergistic effects of the attractants α-pinene and *exo*-brevicomin on the southern and western pine beetle and associated predators in Arizona. J. Appl. Ent. (*in review*)

Kinzer, G. W., A. G. Fentimanjr, T. F. Pagejr, R. L. Foltz, J. P. Vite, and G. B. Pitman. 1969. Bark beetle attractants: Identification, synthesis and field bioassay of a new compound isolated from *Dendroctonus*. Nature 211:477-478.

Libbey, L. M., M. E. Morgan, T. B. Putnam, and J. A. Rudinsky. 1974. Pheromones released during inter- and intra-sex response of the scolytid beetle *Dendroctonus brevicomis*. J. Insect Physiol. 20:1667-1671.

Light, D. M., M. C. Birch, and T. D. Paine. 1983. Laboratory study of intraspecific and interspecific competition within and between two sympatric bark beetle species, *Ips pini* and *I. paraconfusus*. Zeitschrift fur angewandte Entomologie 96:233-241.

Lindgren, B. S. 1983. A multiple funnel trap for scolytid bark beetles (Coleoptera). Can. Entomol. 115:229-302.

Osgood, E. A. J., and E. W. Clark. 1963. Methods of sexing and sex ratios of the southern pine beetle, *Dendroctonus frontalis* Zimm. Can. Entomol. 95:1106-1109.

Payne 2006. http://www.barkbeetles.org/spb/spbbook/Chapt2.html#2.

Payne, T. L. 1975. Bark beetle olfaction. III. Antennal olfactory responsiveness of *Dendroctonus frontalis* Zimmerman and *D. brevicomis* LeConte (Coleoptera: Scolytidae) to aggregation pheromones and host tree terpene hydrocarbons. J. Chem. Ecol. 1:233-242.

Payne, T. L., J. E. Coster, and P. C. Johnson. 1977. Effects of slow-release formulations of synthetic endo- and exo-brevicomin on southern pine beetle flight and landing behavior. Journal of Chemical Ecology 3:133-141.

Payne, T. L., J. E. Coster, J. V. Richerson, L. J. Edson, and E. R. Hart. 1978. Field responses of the southern pine beetle to behavioral chemicals. Environ. Entomol. 7:578-582.

Pitman, G. B., J. P. Vité, G. W. Kinzer, and A. F. F. Jr. 1969. Specificity of population-aggregating pheromones in *Dendroctonus*. J. Insect Physiol. 15:363-366.

Poland, T. M., and J. H. Borden. 1998. Disruption of secondary attraction of the spruce beetle, *Dendroctonus rufipennis*, by pheromones of two sympatric species. J. Chem. Ecol. 24:151-166.

Raffa, K. F., T. W. Phillips, and S. M. Salom. 1993. Strategies and mechanisms of host colonization by bark beetles. Pp. 103-128 *in* T. D. Schowalter and G. M. Filip, eds. Beetle-Pathogen Interactions in Conifer Forests. Academic Press, New York.

Rankin, L. J., and J. H. Borden. 1991. Competitive interactions between the mountain pine beetle and the pine engraver in lodgepole pine. Can. J. For. Res. 21:1029-1036.

Reeve, J. D. 1997. Predation and bark beetle dynamics. Oecologia 112:48-54.

Renwick, J. A. A. 1967. Identification of two oxygenated terpenes from the bark beetles *Dendroctonus frontalis* and *Dendroctonus brevicomis*. Boyce Thompson Inst. 23:355-360.

Ryker, L. C., and L. M. Libbey. 1982. Frontalin in the male mountain pine beetle. J. Chem. Ecol. 8:1399-1409.

SAS. (1991-2000). SAS Institute Inc. SAS/STAT® Users Guide Release Cary, North Carolina.

Silverstein, R. M., R. G. Brownlee, T. E. Bellas, D. L. Wood, and L. E. Brown. 1969. Brevicomin: Principal sex attractant in the frass of the female Western pine beetle. Science 159:889-891.

Sullivan, B. T. 2005. Electrophysiological and behavioral responses of southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae), to volatiles isolated from conspecifics. J. Econ. Entomol. 98:2067-2078.

Sullivan, B. T., W. P. Shepherd, D. S. Pureswaran, and K. Mori. 2007. Evidence that (+)-*endo*-brevicomin is a male produced aggregation pheromone component of the southern pine beetle, *Dendroctonus frontalis*. Journal of Chemical Ecology submitted

Svihra, P., T. D. Paine, and M. C. Birch. 1980. Interspecific olfactory communications in southern pine beetles. Naturwissenschaften:67S.

Vité, J. P., R. F. Billings, C. W. Ware, and K. Mori. 1985. Southern pine beetle: enhancement or inhibition of aggregation response mediated by enantiomers of endo-brevicomin. Naturwissenschaften 72:99.

Vite, J. P., R. I. Gara, and H. D. v. Scheller. 1964. Field response to attractants of bark beetles infesting southern pines. Contributions of the Boyce Thompson Institute 22:461-470.

Vite, J. P., and J. A. A. Renwick. 1971. Inhibition of *Dendroctonus frontalis* response to frontalin by isomers of brevicomin. Naturwissenschaften 58:418.

Wood, D. L. 1982a. The role of pheromones, kairomones and allomones in the host selection and colonization of bark beetles. Annu. Rev. Entomol. 27:411-446.

Wood, S. L. 1982b. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Bringham Young University, Provo, Utah.

Table 1. Compounds, purity, source release rates and devices used in field test.

Turpintine ?/day

Frontalin 5.2mg/day (at 23oC)

Exo-brevicomin 1.7mg/day (at 23oC)**Figure legends**

Figure 1

Pheromone profiles (amount per beetle) of *D. brevicomis* from Arizona and *D. frontalis* from Arizona and Mississippi. Note different scales on Y-axis.

Figure 2

Coupled gas chromatography-electroantennographic detection (GC-EAD) analyses of the antennae of male and female *D. frontalis* to volatiles collected from *exo*-brevicomin baits used in field trapping tests. The upper trace was produced by the flame ionization detector (FID) of the GC. The lower two traces represent the composite EAD traces of either five female or five male beetles. Values associated with each EAD peak represent the mean and standard error of the individual responses (mv). The quantitative proportion of *exo*- to *endo*-brevicomin in the sample was approximately 108:1.

Figure 3

Number of *D. frontalis* captured in baited traps in Mississipi. Bars with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

Figure 4

Number of *Thanasimus dubius* and *Hylastes* sp. captured in baited traps. Bars with the same letter are not significantly different, REGW multiple range test, $P < 0.05$.

Figure 1

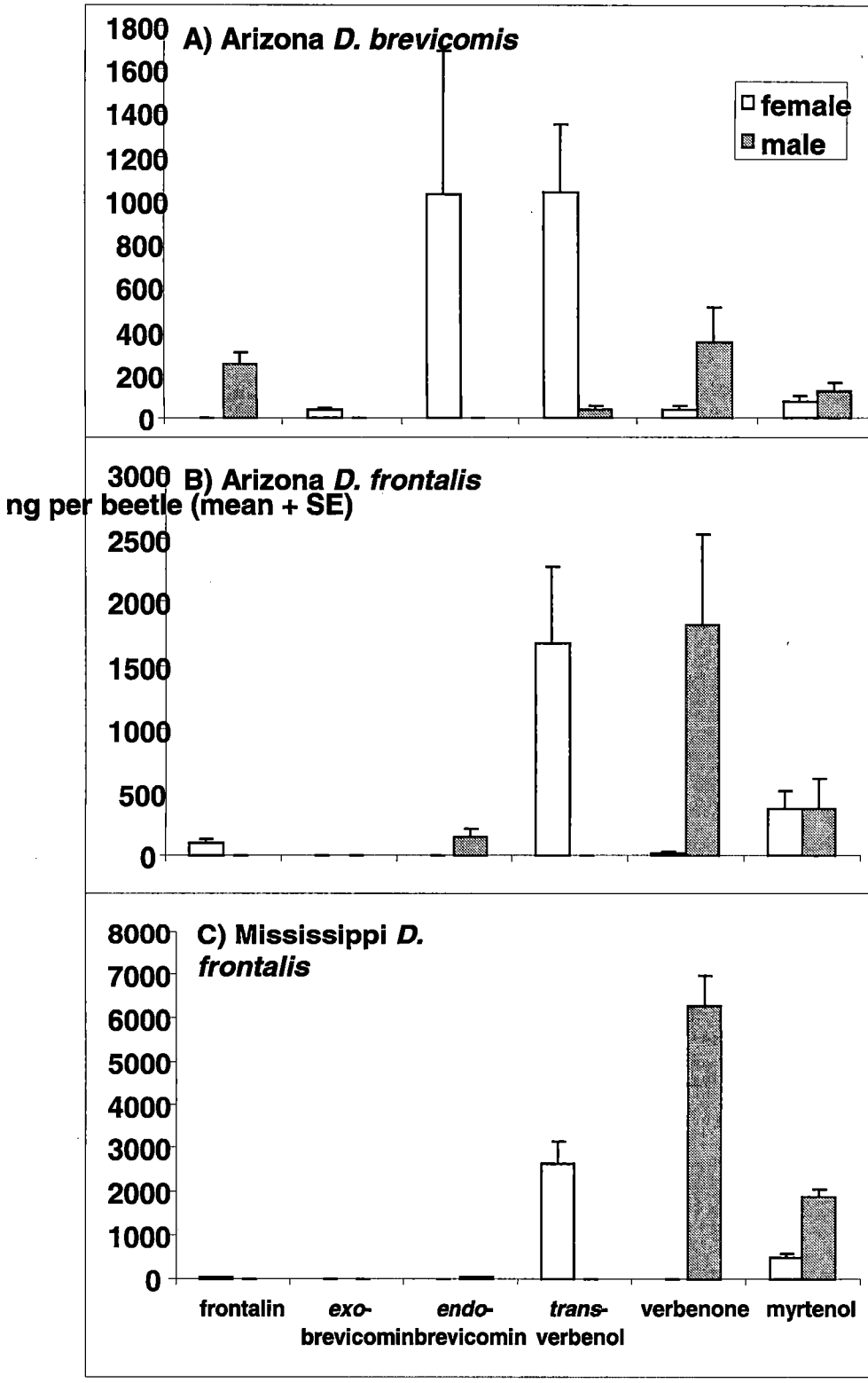

A) Arizona D. brevicomis
female
male
B) Arizona D. frontalis
C) Mississippi D. frontalis
ng per beetle (mean + SE)
frontalin
exo-brevicomin
endo-brevicomin
trans-verbenol
verbenone
myrtenol
pheromone

Figure 2

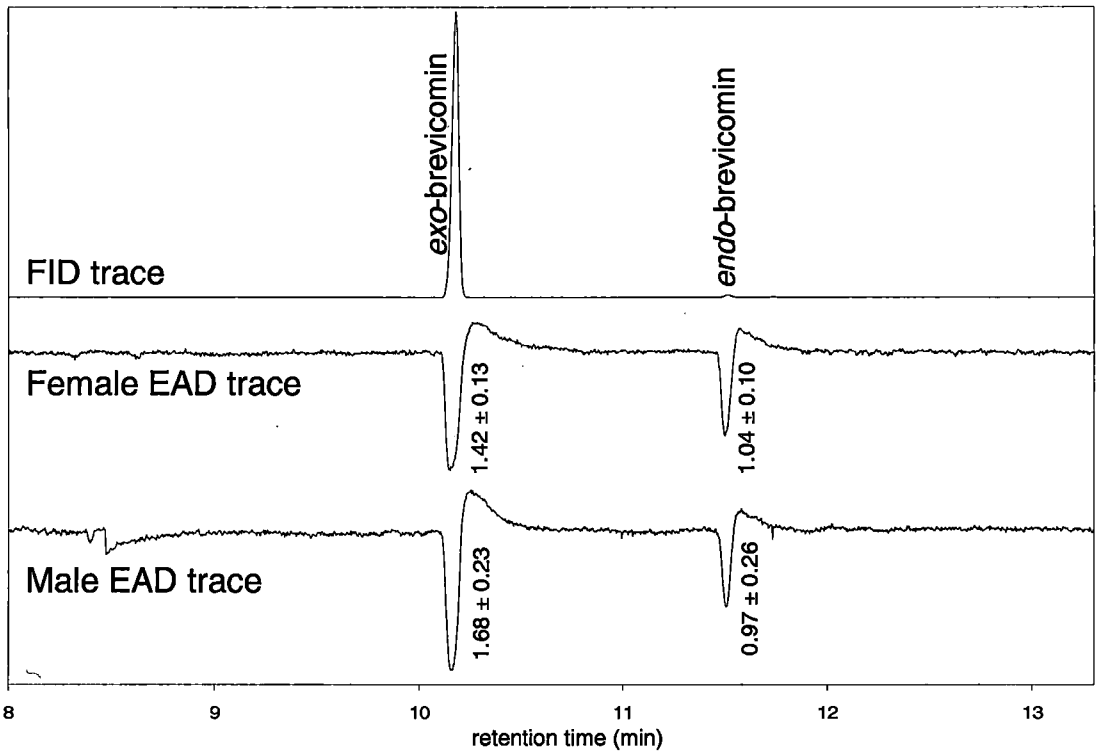

exo-brevicomin
endo-brevicomin
FID trace
Female EAD trace
1.42 ± 0.13
1.04 ± 0.10
Male EAD trace
1.68 ± 0.23
0.97 ± 0.26
8
9
10
11
12
13
retention time (min)

Figure 3

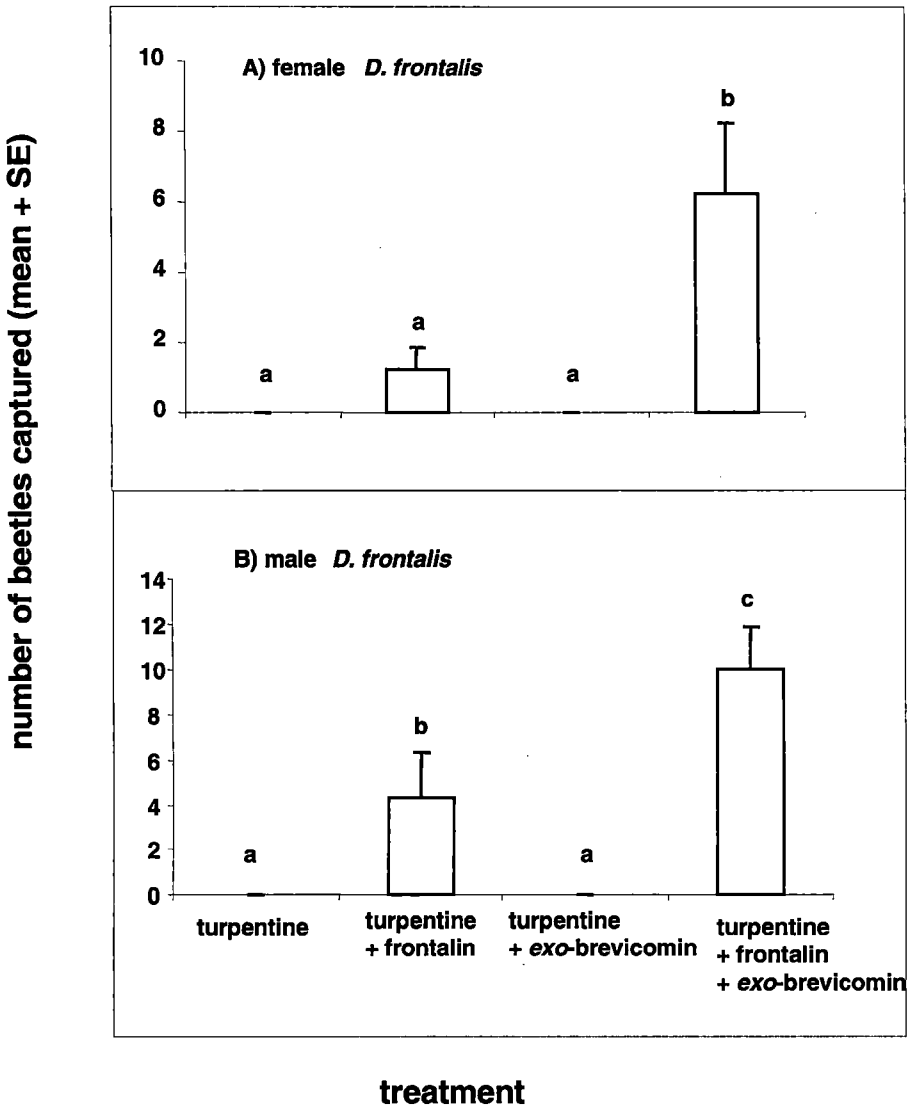
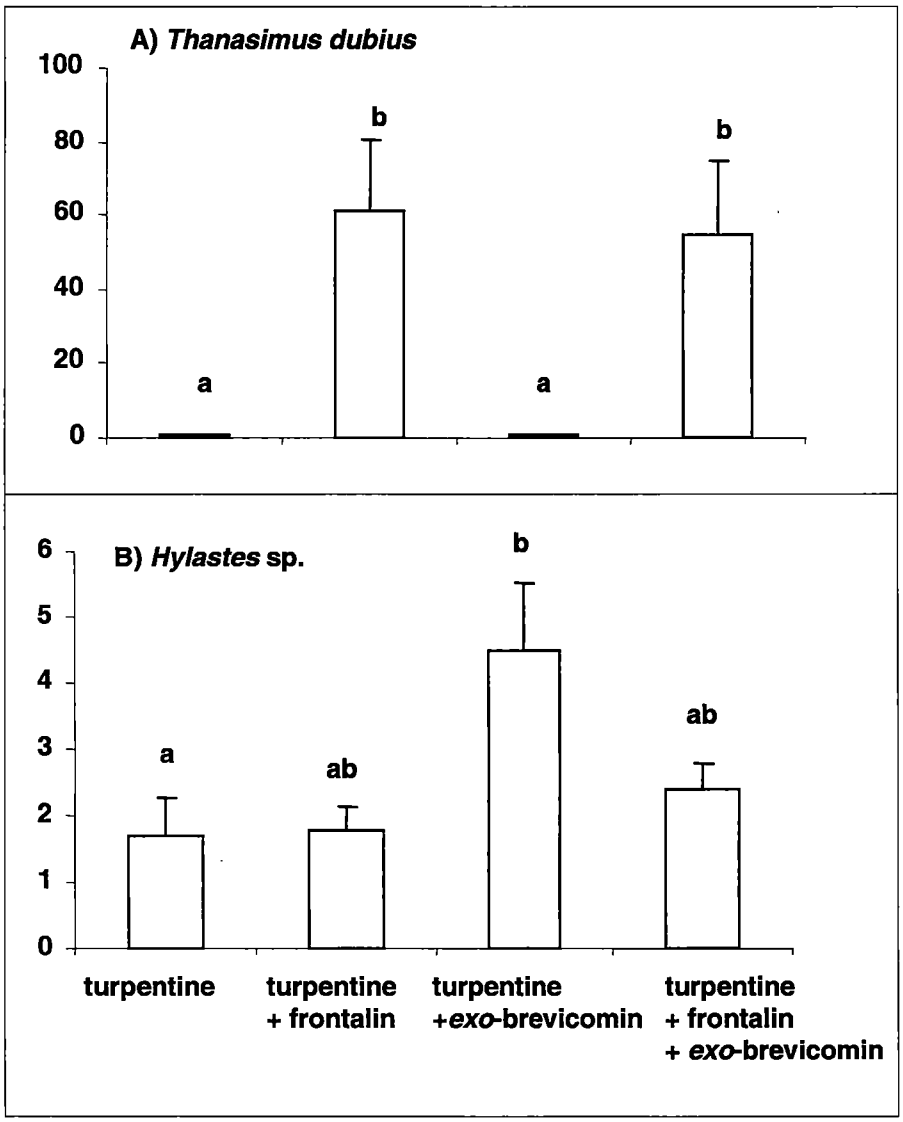

A) female D. frontalis
B) male D. frontalis
number of beetles captured (mean + SE)
treatment
turpentine
turpentine + frontalin
turpentine + exo-brevicomin
turpentine + frontalin + exo-brevicomin

Figure 4


A) Thanasimus dubius
100
80
60
40
20
0
a
b
a
b
B) Hylastes sp.
6
5
4
3
2
1
0
a
ab
b
ab
turpentine
turpentine + frontalin
turpentine +exo-brevicomin
turpentine + frontalin + exo-brevicomin